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Calcium-Dependent Protein Kinase: A Tool for Plants to Crack the Calcium Code

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ABSTRACT

Ca^{2+} signals are involved in most aspects of growth and development of plant, including response to hormone signaling, various biotic and abiotic stresses, germination, cell division, cell expansion, pollen tube growth and fertilization. The calcium-dependent protein kinases (CDPKs) constitute one of the largest Ca^{2+} sensing subfamilies of plant-specific protein kinases that decodes the transient changes of Ca^{2+} concentration in the cytoplasm in response to extrinsic and intrinsic cues. The unique domain structure of CDPKs makes them not only "sensors" but also "responders" to these Ca^{2+} signatures. A multigene family consisting of 34, 31 and 20 genes in *Arabidopsis*, rice and wheat, respectively, encodes CDPKs. The multigenic nature and diverse spatial and temporal differential expression have been reported in many plant species, which emphasizes on the precise role of isoforms in developmental (e.g. pollen tube) as well as stress responsive pathways (e.g. ROS). The regulation of CDPKs has been reported to be at transcriptional and post translational level. The signaling pathways mediated by CDPKs have also been found to overlap with MAP kinase pathways, suggesting of an intricate network, which regulate precise responses of plants. The proteins interacting with CDPKs are diverse in their function (e.g. transcription factor, channel protein, v-SNARE) which indicates that CDPKs play important role in regulating the Ca^{2+} signaling cascade, leading to extremely precise response of plants during development and adaptation to environmental cues. This functional diversity and their cross-talks are being discussed in this review.

Keywords: calcium-dependent protein kinase, cross-talk, development, functional genomics, and stress

Abbreviations: **ABA**, abscisic acid; ABF, ABRE-binding factor; **AM**, arbuscular mycorrhiza; **ATP**, adenosine triphosphate; **BA**, 6-benzyladenine; **$CaCl_2$**, calcium chloride; **CCaMK**, calcium or calcium/calmodulin regulated kinases; **CDPK/CPK**, calcium-dependent protein kinase; **CLD**, calmodulin-like domain; **CRK**, CDPK-related kinase; **GA**, gibberellins; **HR**, hypersensitive response; **HSP**, heat shock protein; **IAA**, indole-3-acetic acid; **JA**, jasmonic acid; **MAPK**, mitogen-activated protein kinase; **NADH**, reduced nicotinamide adenine dinucleotide; **PCD**, programmed cell death; **PR**, pathogenesis-related; **ROS**, reactive oxygen species; **SA**, salicylic acid; **UTR**, untranslated region; **VIGS**, virus-induced gene silencing; **WT**, wild type

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INTRODUCTION

The crucial role of Ca^{2+} in cellular function has been well established since the 19th century (Wyn Jones and Lunt 1967; Helper 2005). In a major breakthrough research, Williamson and Ashley (1982) microinjected photoprotein aequorin in internode cells of *Nitella* and *Chara*, and demonstrated a remarkable rise in the Ca^{2+} level which decreased the cytoplasmic streaming, establishing a firm relationship between the action potential, Ca^{2+} and inhibition of cytoplasmic streaming. Further studies strongly established Ca^{2+} as indispensable secondary messengers in

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Invited Review

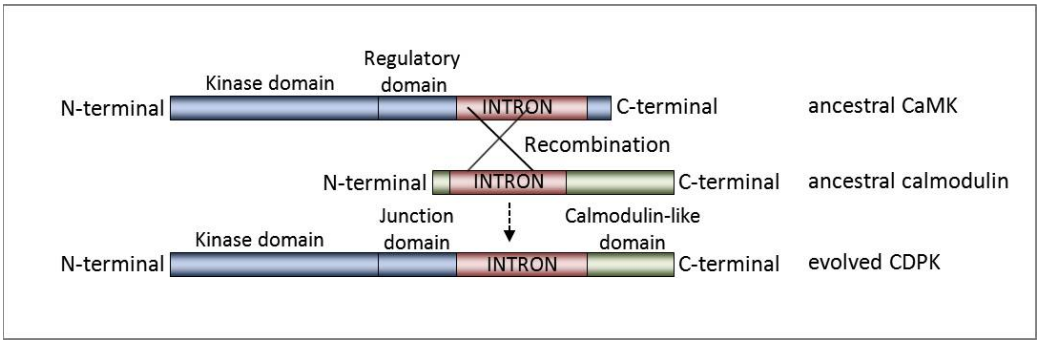

Fig. 1 Origin of CDPK by recombination between CaMK and calmodulin protein. Adapted from Zhang and Choi (2001).

cellular signaling via "calcium decoders" like calmodulins and kinases (reviewed in Sanders *et al.* 1999; Knight 2000; Evans *et al.* 2001; Knight and Knight 2001). While investigating for calcium-dependent and calmoldulin-independent kinases, calcium-dependent protein kinases (CDPKs) were discovered (Putnam-Evans *et al.* 1986; Harmon *et al.* 1987). CDPKs are plant-specific protein kinases found throughout the plant kingdom from algae to angiosperms (Ludwig *et al.* 2004), and even in protozoa (Harmon *et al.* 2001). CDPKs are not "sensor relayer" like calmodulins, but also "sensor responder" through their kinase domain where Ca^{2+} signature is sensed by the calmodulin-like domain (CLD; Harper *et al.* 1991; Harmon *et al.* 2001; Hrabak *et al.* 2003). Harper *et al*. (1991) reported that CDPKs may have evolved as a fusion of two preexisting genes encoding for a Ca^{2+}/calmodulin-dependent kinase with a calmodulin-like gene. In subsequent phylogenetic analysis of CDPK genes with exon/introns, it has been observed that the intron position in the CLD of protist CDPKs are common with animal and fungal calmodulin genes, furthermore, protist and plant CDPKs share introns which originated before the divergence of plants from Alveolates (Zhang and Choi 2001). Hence, the ancestral CDPK gene may have originated from the fusion of protein kinase and calmodulin genes. Plant CDPKs are also found to share monophyletic origin with CDPK-related kinases and phosphoenolpyruvate carboxylase kinases (**Fig. 1**; Zhang and Choi 2001). Several essential cellular and developmental processes in lifecycle of plants are regulated by CDPKs. Modulation of cytoplasmic Ca^{2+} flux due to environmental or internal cues are perceived by CDPKs and transduced to downstream signaling molecules by phosphorylating specific substrate(s). Aspects like hormone signaling, various biotic and abiotic stresses, germination, cell division, cell expansion, stomatal function, pollen tube growth and fertilization (reviewed in Cheng *et al.* 2002; Ludwig *et al.* 2004) are regulated by CDPKs.

In this review, focus is on the transcriptional and post transcriptional regulations in CDPK gene families. Emphasis will be given on the differential expression profile of CDPK gene families in different plant species and their functional analysis in vegetative and reproductive developmental stages, as well as, biotic and abiotic stresses. Furthermore, cross-talk among CDPK mediated signaling pathways, with elaboration on MAPK pathways, will be discussed in detail. Moreover, corroborating expression data with protein-protein interaction and localization analysis of CDPKs will be touched upon for elucidating its role in activating signaling cascade upon sensing internal and external cues.

REGULATION OF CDPK ACTIVITY

Structurally CDPKs consist of four domains (Cheng *et al.* 2002). The N-terminal domain is highly variable and contains myristoylation/palmotylation sites for subcellular targeting (Cheng *et al.* 2002). The kinase domain is the catalytic domain with an ATP binding site, which is followed by an autoinhibitory domain (Harmon *et al.* 1994) and the CLD that contains EF-hands for binding to Ca^{2+} (Cheng *et al.* 2002). A relatively short variable C-terminal domain follows the CLD. CDPKs are activated by binding directly to Ca^{2+} ions (Sanders *et al.* 2002). Ca^{2+} ion binds to the CLD, which triggers a conformational change in this domain leading to intramolecular interaction between CLD and the autoinhibitory domain, resulting in release of the catalytic domain. In the basal state of CDPK, autoinhibitory domain acts as pseudosubstrate and blocks the activity by binding to kinase domain (Reddy 2001). The Ca^{2+} mediated activation model is supported by studies showing removal of the CLD results in an inactive kinase where the activity could be partially rescued by addition of exogenous CLD protein in the presence of Ca^{2+}. Moreover, truncated CDPK protein, without both the CLD and autoinhibitory domain produced a constitutively active kinase (Harmon *et al.* 1994; Harper *et al.* 1994; Huang *et al.* 1996; Yoo and Harmon 1996). In a later study, it has been found that CLD is composed of two globular EF structural domains (N-lobe, C-lobe), each containing a pair of Ca^{2+} binding site. At a low cytosolic level of Ca^{2+}, the C-lobe interacts with the junction, but kinase domain remains in an autoinhibited state. Eventually, with increase in Ca^{2+} level, Ca^{2+} ion binds to N-lobe and triggers the conformational change that leads to activation of the enzyme (Christodoulou *et al.* 2004).

CDPKs comprise of multigene families [34 in *Arabidopsis*, 31 in rice (*Oryza sativa* L.), 20 in wheat (*Triticum* sp.)] where the isoforms show diverse spatial and temporal expression pattern and association with diverse functions. However, the biochemical mechanism of how the CDPK itself is regulated at the post translation stage by (auto)phosphorylation as well as the downstream targets are still a puzzle. *In vitro* autophosphorylation of CDPKs are reported for eight CDPKs from *Arabidopsis* (AtCPK1, AtCPK4, AtCPK5, AtCPK10, AtCPK11, AtCPK16 and AtCPK28), PfCPK1 from *P. falciparum* and CRKs from *Arabidopsis* (AtCRK3 and AtCRK6; Hegeman *et al.* 2006). Thirty-five sites have been detected, out of which, 15 sites are found to be clustered into five conserved groups and distributed in kinase, CLD and N-terminal variable domains. The other 20 sites are not conserved and also found in similar three domains as in the case of conserved sites. Over all, the frequency of sites is higher in the variable N-terminal domain; however, none of the phosphorylation sites are observed to be in the junction domain (Hegeman *et al.* 2006). Autophosphorylation sites have also been mapped for tomato (*Solanum lycopersicum* L.) CDPK1 (Rutschmann *et al.* 2002; Chang *et al.* 2011), tobacco (*Nicotiana tabacum* L.) NtCPK2 (Glinski *et al.* 2003) and ice plant (*Mesembryanthemum crystallinum* L.) McCPK1 (Chehab *et al.* 2004). The functions of variable N-terminal domain, regulation of activity as well as substrate specificity of CDPKs were elusive for quite some time. Activity of LeCPK2, expressing in flowers and responding to heat/cold stress, mechanical wounding and phytohormones (ethylene, MJ, and SA), has been reported to be dependent on the 161 residue of CLD (Chang *et al.* 2009, 2011). Stress-inducible phosphorylation in N-terminal domain have been found to be exclusively located in NtCDPK2 and NtCDPK3, where phosphorylation

is differential (serine-40/threonine-65 in NtCDPK2 and serine-54 in NtCDPK3) despite 91% overall sequence identity. Domain swap as well as mutation in the myristoylation and palmitoylation site experiments established the exclusivity of regulation of the respective N-terminal domain (Witte *et al.* 2010). Variable N-terminal domain mediated substrate specificity has also been reported for NtCDPK1. NtCDPK1 phosphorylates Ser-114 of RSG (Repression of Shoot Growth), a transcriptional activator regulating endogenous GA content, and facilitates its binding to 14-3-3 proteins. This results in sequestering of RSG in the cytoplasm, hence inhibiting GA biosynthesis (Fukazawa *et al.* 2000; Igarashi *et al.* 2001; Ishida *et al.* 2004, 2008). Mutation at R10A in the N-terminal domain of NtCDPK1 reduces RSG recognition. Moreover, chimeric AtCPK9 with N-terminal domain of NtCDPK1 phosphorylates RSG as NtCDPK1, although native AtCPK9 neither binds nor phosphorylates RSG (Ito *et al.* 2010). SOS1 (plasma membrane sodium/proton exchanger Salt-Overly-Sensitive 1) is also reported to be relieved from autoinhibition upon phosphorylation of the autoinhibitory domain by SOS2-SOS3 (calcium-dependent protein kinase complex; Quintero *et al.* 2011). A plastid glutamine synthetase of *Medicago truncatula* (MtGS2) is regulated by phosphorylation at Ser-97, catalyzed by a CDPK. On phosphorylation of this residue, a 14-3-3-binding motif is created which allows the formation of the GS2-14-3-3 complex. This complex is further recognized by an unknown plant protease that cleaves the enzyme (Lima *et al.* 2006a). Interestingly, in a subsequent article, it is also found that GS1, another isoenzyme of GS2, is phosphorylated by CDPK. However in contrast to the earlier report, the phosphorylated GS1 does not interact with 14-3-3 proteins (Lima *et al.* 2006b). These findings strongly indicate towards the complexity involved in post-transcriptional regulation of isoezymes mediated by phosphorylation. Even differential activity of *Arabidopsis* AtCPK21 is found to be regulated by EF motifs. The N-terminal EF1- and EF2-motifs and C-terminal EF3- and EF4-motifs show varying contribution to Ca^{2+}-regulated kinase activity. The N-terminal EF-hand pair has been reported to control specificity of AtCPK21 function (Franz *et al.* 2011). Even sensitivity of homologous CDPKs to Ca^{2+} is found to confer functional specificity as observed in chickpea (*Cicer arietinum* L.). Both CaCPK1 and CaCPK2 transcripts and proteins are abundant in roots but occur in minor quantities in leaves and stems. CaCPK2 protein and its activity are almost undetectable in flowers and fruits. BA increases both CaCPK1 and CaCPK2 transcripts, proteins and their activities. GA induces accumulation of CaCPK2 transcript and protein but CaCPK1 remains unaffected. The expression of CaCPK1 shows response to biotic stress and CaCPK2 is found to be responsive to dehydration stress (Syam and Chelliah 2006a). These two CDPKs show significant variations in their biochemical properties as well as Ca^{2+} sensitivities suggesting that they might be playing divergent role in signaling (Syam and Chelliah 2006b).

VEGETATIVE PHASE DEVELOPMENT AND CDPKs

Specialized vegetative tissues

The first report on calcium-dependent/calmodulin-independent protein kinase activity was in pea (*Pisum sativum* L.) extracts (Hetherington and Trewavas 1982). Since then, many CDPKs have been reported to be involved in developmental processes and specialized diverse functions (**Table 1**; Ludwig *et al.* 2004). During vegetative development, regulation of functions in specialized cells like guard cells, xylem and phloem, as well as complex signaling processes like light regulation and symbiosis, show involvement of CDPKs. Transcripts of *GhCPK1*, from cotton (*Gossypium hirsutum*) are found to preferentially accumulate in the elongating fiber (Huang *et al.* 2008), further study shows that cotton ACS2 activity increases on phosphorylation by GhCPK1, pointing to the possibility that GhCPK1 is involved in cotton fiber elongation via ACS2 (Wang *et al.* 2011). Involvement of nitric oxide and cGMP in auxin response during adventitious root formation in cucumber (*Cucumis sativus* L.) is well established (Pagnussat *et al.* 2002). Lanteri *et al*. in (2006) reported that Ca^{2+} and CDPK activity are downstream messengers in the signalling pathway triggered by auxins and nitric oxide to promote adventitious root formation.

Sieve elements and companion cell complex is integrative part of translocation of specific population of transcripts and proteins to distant organs. Of the five kinases isolated from phloem sap extracted from stem of pumpkin (*Cucurbita maxima*), two are CDPKs. *CmCPK1* has been cloned using peptide microsequences and further amino-terminal sequencing reveals that an amino-terminally cleaved form of CmCPK1 exists in phloem sap. On the other hand, CmCPK2 has been detected in companion cells. Therefore, these two isoforms could be involved in the control of ribonucleoprotein complex exchange through plasmodesmata between sieve elements and companion cell (Yoo *et al.* 2002). There has been a recent report showing engineering of heterologous, *AtCPK1*, in *Rubia cordifolia* cells, which increases production of anthraquinone content. They also report of a positive correlation between enhanced anthraquinone biosynthesis and activation of isochorismate synthase gene expression (Shkryl *et al.* 2011). It is also reported that *AtCPK1* acts on secondary metabolism via the activation of ROS production (Bulgakov *et al*. 2011). This report opens up a new dimension in engineering CDPK genes for enhanced secondary metabolite production.

Light regulation

Involvement of CDPK orthologs in light regulated pathways is being reported in multiple plant species. The expression of *OsCDPK2* in rice (*Oryza sativa* L.) plants has been found to decrease under low light condition in leaves and significantly increase under dark condition (Frattini *et al*. 1999). In accord with this observation, OsCDPK2 protein has been found to be almost undetectable in light exposed leaves, but accumulated in high quantity after incubation in dark. Even a significant decrease in *OsCDPK2* mRNA content has been observed in etiolated coleoptiles on exposure to light (Breviario *et al*. 1995). When OsCPK2 is overexpressed in rice, even then OsCDPK2 protein was barely detectable in leaves exposed to light, in transgenic as well as control rice plants. Hence, these findings strongly demonstrate that *OsCDPK2* transcript accumulation as well as protein stability is controlled by light (Morello *et al.* 2000). However, OsCPK2 promoter-leader region is observed to be constitutive and independent of light or dark condition. But on inserting the 3` UTR region, light-regulated expression could be restored, indicating that light dependent regulation is mediated by a mechanism driven by the 3`UTR. Regulation of other CDPKs by light has been described in maize (*Zea mays* L.; Estruch *et al*. 1994) and in zucchini (*Cucurbita pepo* L.; Ellard-Ivey *et al*. 1999). *CsCDPK3*, isolated from cucumber, shows an organ dependent differential expression regulated by light as well as hormone. Significant transcript accumulation is reported in dark grown hypocotyl followed by root and cotyledon. Moreover, exposure to light downregulates *CsCDPK3* in hypocotyl, however, on light treatment, transcripts accumulate in cotyledon tissue. Even on exogenous application of cytokinin to etiolated tissue, *CsCDPK3* is upregulated in cotyledons and downregulated in roots (Ullanat and Jayabaskaran 2002). In a recent report, StCDPK2 is reported to be highly expressed in leaves and green sprouts. Moreover, it shows differential regulation under light treatment. Promoter region reveals the presence of light responsive *cis*-acting elements, which corresponds well with the expression profile (Giammaria *et al.* 2011).

Table 1 Functionally characterized CDPK genes across plant species. The table has been sorted according to alphabetical order of species, then by gene name followed by chronology of references.

Gene name	Species	Characterization	Reference
AtCPK1	*Arabidopsis thaliana*	Enhances NADPH activity on overexpression	Xing *et al*. 2001
AtCPK1	*A. thaliana*	Mediates pathogen resistance	Coca and San Segundo 2010
AtCPK1	*A. thaliana*	Heterologous expression of *AtCPK1* in *Rubia cordifolia* cells increases anthraquinone content	Shkryl *et al*. 2011
AtCPK1	*A. thaliana*	Secondary metabolism via the activation of ROS production	Bulgakov *et al.* 2011
AtCPK3	*A. thaliana*	Expresses in the guard cells, functions in guard cell ion channel regulation	Mori *et al*. 2006
AtCPK3	*A. thaliana*	Salt stress acclimation	Mehlmer *et al*. 2010
AtCPK4	*A. thaliana*	Overexpression increases ABA sensitivity and salt hypersensitivity in seedling growth and affects stomatal regulation	Zhu *et al*. 2007
AtCPK4	*A. thaliana*	Involved in primary responses in innate immune signaling	Boudsocq *et al*. 2010
AtCPK5	*A. thaliana*	Involved in primary responses in innate immune signaling	Boudsocq *et al*. 2010
AtCPK6	*A. thaliana*	Expresses in the guard cells, functions in guard cell ion channel regulation	Mori *et al*. 2006
AtCPK6	*A. thaliana*	Involved in primary responses in innate immune signaling	Boudsocq *et al*. 2010
AtCPK6	*A. thaliana*	Overexpressing plants confers tolerance to salt/drought stresses	Xu *et al*. 2010
AtCPK6	*A. thaliana*	Positive regulator of methyl jasmonate signaling in guard cells	Munemasa *et al*. 2011
AtCPK9	*A. thaliana*	N-terminal domain swapped with NtCDPK1 and the chimeric AtCDPK phosphorylates RSG	Ito *et al*. 2010
AtCPK10	*A. thaliana*	Drought and salt stress responsive	Urao *et al*. 1994
AtCPK10	*A. thaliana*	Activates stress and ABA inducible promoter	Sheen 1996
AtCPK10	*A. thaliana*	Abscisic acid and Ca^{2+}-mediated stomatal regulation in response to drought stress	Zou *et al*. 2010
AtCPK11	*A. thaliana*	Drought and salt stress responsive	Urao *et al*. 1994
AtCPK11	*A. thaliana*	Interaction with *AtDi19*	Milla *et al*. 2006b
AtCPK11	*A. thaliana*	Overexpression increases ABA sensitivity and salt hypersensitivity in seedling growth and affects stomatal regulation	Zhu *et al*. 2007
AtCPK11	*A. thaliana*	Involved in primary responses in innate immune signaling	Boudsocq *et al*. 2010
AtCPK12	*A. thaliana*	Negatively regulates ABA signaling	Zhao *et al*. 2011
AtCPK14	*A. thaliana*	High expression in pollen	Becker *et al*. 2003
AtCPK14	*A. thaliana*	High expression in pollen	Harper *et al*. 2004
AtCPK16	*A. thaliana*	High expression in pollen	Harper *et al*. 2004
AtCPK17	*A. thaliana*	High expression in pollen	Harper *et al*. 2004
AtCPK17	*A. thaliana*	Polarization of pollen tube	Myers *et al*. 2009
AtCPK18	*A. thaliana*	High expression in pollen	Becker *et al*. 2003
AtCPK20	*A. thaliana*	High expression in pollen	Becker *et al*. 2003
AtCPK21	*A. thaliana*	Regulates guard cell anion channel SLAC1	Geiger *et al*. 2010
AtCPK21	*A. thaliana*	Abiotic stress response	Franz *et al*. 2011
AtCPK23	*A. thaliana*	Responses to drought and salt stresses via stomatal closure	Ma and Wu 2007
AtCPK23	*A. thaliana*	Regulates guard cell anion channel SLAC1	Geiger *et al*. 2010
AtCPK24	*A. thaliana*	High expression in pollens	Becker *et al*. 2003
AtCPK24	*A. thaliana*	High expression in pollens	Harper *et al*. 2004
AtCPK26	*A. thaliana*	High expression in pollens	Becker *et al*. 2003
AtCPK30	*A. thaliana*	Activates stress and ABA inducible promoter	Sheen 1996
AtCPK32	*A. thaliana*	Induced by ABA and salt stress	Choi *et al*. 2005
AtCPK32	*A. thaliana*	Induced by touch, wounding, NaCl and darkness	Chotikacharoensuk *et al*. 2006
AtCPK34	*A. thaliana*	High expression in pollen	Harper *et al*. 2004
AtCPK34	*A. thaliana*	Polarization of pollen tube	Myers *et al*. 2009
AhCDPK1	*Arachis hypogaea* (peanut)	Seed development	Jain *et al*. 2011
AhCPK2	*A. hypogaea*	Responds to drought stress	Raichaudhuri *et al*. 2006
CaCPK1	*Cicer arietinum* (chickpea)	Expressed abundantly in roots and induced by biotic stress	Syam and Chelliah 2006a
CaCPK2	*C. arietinum*	Expressed abundantly in roots and responsive to GA as well as dehydration stress	Syam and Chelliah 2006a
CaCDPK3	*Capsicum annuum* (pepper)	Induced by ABA, salicylic acid, jasmonic acid, ethephon	Chung *et al*. 2004
CsCDPK3	*Cucumis sativus* (cucumber)	Cytokinin and light regulated expression	Ullanat and Jayabaskaran 2002
CsCDPK5	*C. sativus*	Induced by Cytokinin, IAA, ABA, GA	Kumar *et al*. 2004
CpCPK1	*Cucurbita maxima* (pumpkin)	Expresses in etiolated tissue	Ellard-Ivey *et al*. 1999
FaCDPK1	*Fragaria* x *ananassa* (strawberry)	Expresses in developing fruit and low temperature stress	Llop-Tous *et al*. 2002
GhCPK1	*Gossypium hirsutum* (cotton)	Associates with fiber elongation	Huang *et al*. 2008
GhCPK1	*G. hirsutum*	Regulates cotton fiber growth by phosphorylating ACS2	Wang *et al*. 2011
HbCDPK1	*Hevea brasiliensis* (rubber)	Preferential transcript accumulation in latex as well as under mechanical wounding, jasmonic acid (JA) and ethephon	Zhu *et al*. 2010
HvCDPK1	*Hordeum vulgare* (barley)	Mediates GA response and alters vacuole function	McCubbin *et al*. 2004
HvCDPK2	*H. vulgare*	Constitutively active in aleurone layer	McCubbin *et al*. 2004
HvCDPK3	*H. vulgare*	Constitutive active expression, compromised penetration, resistance to powdery mildew	Freymark *et al*. 2007
HvCDPK4	*H. vulgare*	Constitutive active expression, compromised penetration, resistance to powdery mildew	Freymark *et al*. 2007
IiCPK2	*Isatis indigotica* (indigowood)	Induced by salinity, cold stress and GA treatment	Lu *et al*. 2006
MsCK1	*Medicago sativa* (alfalfa)	Induced by cold stress	Monroy and Dhindsa 1995
MsCK2	*M. sativa*	Induced by cold stress	Monroy and Dhindsa 1995
MsCPK3	*M. sativa*	Induced by 2,4-D, heat stress	Davletova *et al*. 2001

Table 1 (Cont.)

Gene name	Species	Characterization	Reference
MtCDPK1	*Medicago trancatula* (barrel medic)	Mediates root hair and root cell growth and controls cell wall synthesis	Ivashuta *et al*. 2005
MtCPK3	*M. trancatula*	Expression found in the early stage of nodulation, silencing with RNAi results in nodule number increase	Gargantini *et al*. 2006
McCPK1	*Mesembryanthemum crystallinum* (ice plant)	Responsive to drought and salt stress	Patharkar and Cushman 2000
McCPK1	*M. crystallinum*	Subcellular localization affected by salt and water deficit condition	Chehab *et al*. 2004
McCPK1	*M. crystallinum*	Interaction with v-SNARE family protein	Patharkar and Cushman 2006
McCPK1	*M. crystallinum*	Interaction with McCAP1 a novel coiled-coil protein	Patharkar and Cushman 2006
NtCDPK1	*Nicotiana tabacum* (tobacco)	Induced by Ca^{2+}, GA, ABA, cytokinin, methyl jasmonate, wounding, fungal elicitors, chitosan, salt stress	Yoon *et al*. 1999
NtCDPK1	*N. tabacum*	Interacts with NtRpn3 regulatory subunit of 26S proteosome and regulates cell division, differentiation and cell death	Lee *et al*. 2003
NtCDPK1	*N. tabacum*	Phosphorylates RSG and mediates GA biosynthesis	Ishida *et al*. 2008
NtCDPK2	*N. tabacum*	Induced by fungal elicitor, osmotic stress	Romeis *et al*. 2001
NtCDPK2	*N. tabacum*	Induced by fungal elicitor, osmotic stress	Ludwig *et al*. 2005
NtCDPK3	*N. tabacum*	Induced by fungal elicitor, osmotic stress	Romeis *et al*. 2001
NtCPK4	*N. tabacum*	Accumulates on stigma surface, during the early development of anthers and also induces on GA treatment and under salt stress	Zhang *et al*. 2005
OsCPK4	*Oryza sativa* (rice)	Induced by AM fungus *Glomus intraradices* in rice roots	Campos-Soriano *et al*. 2011
OsCPK7	*O. sativa*	Expresses in seeds	Breviario *et al*. 1995
OsCPK7	*O. sativa*	The protein accumulates in early flower development and late seed development. The transcripts are also negatively regulated by light	Frattini *et al*. 1999
OsCPK7	*O. sativa*	Induced by cold and GA	Yang *et al*. 2003
OsCPK7	*O. sativa*	Induced by JA	Akimoto-Tomiyama *et al*. 2003
OsCPK7	*O. sativa*	Overexpression shows cold tolerance, silencing results in dwarf phenotype	Abbasi *et al*. 2004
OsCPK7	*O. sativa*	Overexpression shows cold tolerance and CRTintP1 and calreticulin, also confers cold tolerance to rice	Komatsu *et al*. 2007
OsCPK9	*O. sativa*	Responds to rice blast treatment	Asano *et al*. 2005
OsCPK12	*O. sativa*	Tolerance to salt stress and reduce resistance to blast disease	Asano *et al*. 2011
OsCPK13	*O. sativa*	Responds to cold, salt and dehydration	Sajio *et al*. 2000
OsCPK13	*O. sativa*	Expresses predominantly in vascular tissue of crown and roots, vascular bundle and central cylinder. Transforments overexpressing OsCPK13 shows similar localization pattern with stronger signal	Sajio *et al*. 2001
OsCPK13	*O. sativa*	Induced by fungal elicitor	Akimoto-Tomiyama *et al*. 2003
OsCPK13	*O. sativa*	Overexpression in *Sorghum* give lesion mimic phenotype and upregulation of pathogen related proteins	Mall *et al*. 2011
OsCPK15	*O. sativa*	Induced by fungal elicitor	Akimoto-Tomiyama *et al*. 2003
OsCPK18	*O. sativa*	Induced by AM fungus *Glomus intraradices* in rice roots	Campos-Soriano *et al*. 2011
OsCPK19	*O. sativa*	Expresses in flower and seed and transcript level declines in presence of light	Breviario *et al*. 1995
OsCPK19	*O. sativa*	The protein accumulates on onset of flowering and seed development and declines with seed maturation	Frattini *et al*. 1999
OsCPK19	*O. sativa*	Constitutive overexpression results in sterility	Morello *et al*. 2000
OsCPK19	*O. sativa*	Promoter delineated	Morello *et al*. 2006
OsCPK20	*O. sativa*	Induced by fungal elicitor	Akimoto-Tomiyama *et al*. 2003
OsCPK21	*O. sativa*	Confers salt tolerance	Asano *et al*. 2011
OsCPK23	*O. sativa*	Expresses in developing seeds	Kawasaki *et al*. 1993
OsCPK23	*O. sativa*	Expressed in mature seeds	Kawasaki *et al*. 1999
OsCPK23	*O. sativa*	Silencing leads to less accumulation of starch	Asano *et al*. 2002
OsCPK23	*O. sativa*	Induced by JA	Akimoto-Tomiyama *et al*. 2003
OsCPK23	*O. sativa*	Antisence SPK transformants shows defective production of storage starch but accumulation of sucrose in watery seeds	Shimada *et al*. 2004
OsCPK24	*O. sativa*	Induced by fungal elicitor	Akimoto-Tomiyama *et al*. 2003
OsCPK24	*O. sativa*	Cytoplasmic localization and biochemical properties	Zhang *et al*. 2005
OsCPK29	*O. sativa*	Expresses in pollen and anther walls	Gupta *et al*. 2007
PiCDPK1	*Petunia* sp.	Mediates pollen tube growth	Yoon *et al*. 2006
PiCDPK2	*Petunia* sp.	Mediates pollen tube growth	Yoon *et al*. 2006
PaCDPK1	*Phalaenopsis amabilis* (moon orchid)	Induces under cold, wounding stress and pathogen attack	Tsai *et al*. 2007
PgCDPK1b	*Panax ginseng* (ginseng)	Repressed by salt stress	Kiselev *et al*. 2009
PgCDPK1c	*P. ginseng*	Induced by salt stress	Kiselev *et al*. 2009
PgCDPK2c	*P. ginseng*	Induced by salt stress	Kiselev *et al*. 2009
PgCDPK3a	*P. ginseng*	Repressed by salt stress	Kiselev *et al*. 2009
PgCDPK4a	*P. ginseng*	Induced by salt stress	Kiselev *et al*. 2009
StCDPK1	*Solanum tuberosum* (potato)	Expression increases in induced stolon	Raices *et al*. 2001
StCDPK1	*S. tuberosum*	Mediates GA signaling during potato tuberization	Gargantini *et al*. 2009
StCDPK2	*S. tuberosum*	Expression in leaf tissue	Ullao *et al*. 2002
StCDPK2	*S. tuberosum*	Involves in light signaling	Giammaria *et al*. 2011
StCDPK3	*S. tuberosum*	Expression specific to early stage of stolon development	Raices *et al*. 2003

Table 1 (Cont.)

Gene name	Species	Characterization	Reference
StCDPK4	*S. tuberosum*	Response to fungal elicitor and phosphorylates downstream St RBOHB (Respiratory Burst Oxidase Homolog) resulting in ROS production	Kobayashi *et al*. 2007
StCDPK5	*S. tuberosum*	Response to fungal elicitor and phosphorylates downstream St RBOHB (Respiratory Burst Oxidase Homolog) resulting in ROS production	Kobayashi *et al*. 2007
RiCDPK2	*S. tuberosum*	*A. solani* stimulated activity of *RiCDPK2* in the host suppress hypersensitive cell death	Hassan *et al.* 2012
SwCPK	*Santalum album* (sandalwood)	Involved in embryogenesis, seed development, germination	Anil and Rao 2001
SwCPK	*S. album*	Associated with oil bodies	Anil *et al*. 2003
LeCDPK1	*Solanum lycopersicum* (tomato)	Induced by fungal elicitor, H_2O_2, wounding	Chico *et al*. 2002
LeCPK2	*S. lycopersicum*	Expresses during flower development, wounding and phytohormone treatment	Chang *et al*. 2009
TaCDPK1	*Triticum aestivum* (wheat)	Transcript accumulation under sucrose treatment	Martínez-Noël *et al*. 2007
VfCPK1	*Vicia faba* (broad bean)	Transcriptionally upregulated by drought ABA and $CaCl_2$	Liu *et al*. 2006
VrCPK1	*Vigna radiata* (mung bean)	Induced by IAA treatment, mechanical strain and salt stress	Botella *et al*. 1996
ACPK1	*Vitis vinifera* (grape vine)	Overexpression in *Arabidopsis* causes higher vigor of plant growth and ABA hypersensitivity in seed germination, seedling growth and stomatal regulation	Yu *et al*. 2007
ZmCDPK	*Zea mays* (maize)	Mediates pollen tube elongation	Estruch *et al*. 1994
ZmCPK1	*Z. mays*	Cold stress responsive	Berberich and Kusano 1997
ZmCPK7	*Z. mays*	Suppression of expression on exposure to white light	Saijo *et al*. 1997
ZmCPK9	*Z. mays*	Suppression of expression on exposure to white light	Saijo *et al*. 1997
ZmCPK10	*Z. mays*	Fungal infection and fungal elicitor	Murillo *et al*. 2001
ZmCPK11	*Z. mays*	Responds to wounding stress and control vacuolar function	Szczegielniak *et al*. 2005
ZmCPK11	*Z. mays*	Component of touch- and wound-induced pathway(s)	Szczegielniak *et al.* 2012

Symbiosis

Symbiotic association of plant-bacteria/mycorrhiza is a specialized process where the plant identifies them as symbiont and not pathogen. Initiation of symbiosis triggers a Ca^{2+} oscillation signal in the root hair as an early event of recognition, so involvement of CDPKs in symbiosis does not come as a surprise (Ehrhardt *et al.* 1996). Silencing of *CDPK1* gene of *M. truncatula* shows significant reduction in root hair and root cell length as well as diminution of rhizobial and mycorrhizal symbiotic colonization, suggesting involvement of CDPK genes in symbiotic association. It is also observed that inactivation of CDPK1 affected actin cytoskeleton organization, accumulation of ROS, induction of genes involved in cell wall development, defense, and hormone metabolism, hence, each of these processes could be responsible for the symbiotic phenotype (Ivashuta *et al.* 2005). Conversely, *MtCPK3,* when silenced, shows increased number of nodules (Gargantini *et al.* 2006), indicating both positive and negative regulation of nodule formation by two isoforms, respectively. In rice, *OsCPK18* and *OsCPK4* are upregulated in response to inoculation with the AM fungus, *Glomus intraradices*. *OsCPK18* expresses precisely in cortical cells of *G. intraradices*-inoculated rice roots (Campos-Soriano *et al.* 2011). These evidences very firmly suggest of CDPKs involvement identifying the early signals of symbiotic association.

REPRODUCTIVE DEVELOPMENT AND CDPKs

Pollen

Growing pollen tubes require a gradient of free Ca^{2+} ions in the cytosol, with oscillating Ca^{2+} flux at the growing apex (Rudd and Franklin-Tong 2001). The oscillation of free Ca^{2+} ions in cytosol is found to be approximately in phase with the oscillation of growth (Holdaway-Clarke *et al.* 1997; Messerli and Robinson 1997). Even signals transduced via Ca^{2+} have role in polarization of pollen tube towards ovule for fertilization (Taylor and Hepler 1997). A maize pollen CDPK is found to express specifically during late stages of pollen development and its protein accumulated during pollen germination. On addition of antisense oligonucleotides directed against CDPK mRNA, impairment of pollen germination and pollen tube growth is observed, suggesting its involvement in pollen germination (Estruch *et al.* 1994). *PiCDPK1* and *PiCDPK2* of *Petunia* are also found to express in pollen and pollen tubes. Transient overexpression or expression of catalytically modified *PiCDPK1* has been performed which results in extremely short tubes with almost spherical tips due to loss of pollen tube growth polarity. On the other hand, expression of catalytically modified *PiCDPK2* inhibits extension of pollen tubes, giving rise to short tubes (Yoon *et al.* 2006). Of the 34 CPKs in *Arabidopsis*, 8 of them (*AtCPK14*, *AtCPK16*, *AtCPK17*, *AtCPK18*, *AtCPK20*, *AtCPK24*, *AtCPK26* and *AtCPK34*) show significant expression in pollen (Becker *et al.* 2003; Harper *et al.* 2004; Honys and Twell 2004). *AtCPK17* and *AtCPK34* are further found to regulate polarization of pollen tube growth (Myers *et al.* 2009). Double disruption mutant of *AtCPK17* and *AtCPK34* (*cpk17*/*cpk34*) shows three-fold reduction of pollen tube growth rate and loss in ability to locate and fertilize ovule. The double mutants also show 350-fold reduction in pollen transmission efficiency, where the phenotype could be rescued on pollen specific expression of *AtCPK34* (Myers *et al.* 2009). Among the rice 31 CDPK genes, seven genes (*OsCPK3*, *OsCPK6*, *OsCPK14*, *OsCPK22*, *OsCPK25/26* and *OsCPK29*) expresses preferentially during the P6 (mature anther just before anthesis) stages of panicle development (Ray *et al.* 2007) and 11 CPKs (*OsCPK2*, *OsCPK6*, *OsCPK11*, *OsCPK14*, *OsCPK17*, *OsCPK21*, *OsCPK22*, *OsCPK25/26*, *OsCPK27* and *OsCPK29*) preferentially expresses in five stages (uninucleate microspore, bicellular pollen, tricellular pollen, mature pollen and germinated pollen) of rice pollen development (Wei *et al.* 2010). Transcript accumulation of *OsCPK2*, *OsCPK21*, *OsCPK22* and *OsCPK29* is significantly high (average raw intensity value in microarray experiment ranged from 169500-190511) in mature and germinated pollen. *OsCPK6* is the only CPK showing maximum transcript accumulation in tricellular pollen stage (average raw intensity value 10551) with gradual decrease with pollen maturity (average raw intensity value 3742) and germination (average raw intensity value 1634; Wei *et al.* 2010).

Interestingly, in phylogenetic analysis of rice CDPKs with other plant species CDPK amino acid sequences, *OsCPK2*, *OsCPK14* and *OsCPK25*/*26* are found to be clustered (IIa clade) with *PiCDPK1*, *AtCPK17*, *AtCPK34* and maize CDPK1, which are already known to be involved in

pollen development (Estruch *et al.* 1994; Yoon *et al.* 2006; Myers *et al.* 2009). In clade IIIa, *OsCPK21*, *OsCPK22* and *OsCPK29* are grouped with *AtCPK24* and *PiCDPK2*, where the latter two genes are already reported to be involved in pollen development (Becker *et al.* 2003; Harper *et al.* 2004; Yoon *et al.* 2006; Myers *et al.* 2009). In subsequent studies, this structural similarity is found to be reflected in functional relatedness where *OsCPK29* is reported to be expressing in pollen as well as to some extent in anther walls (Gupta *et al.* 2007). *OsCPK25/26* (IIa clade) expresses predominantly in mature pollen and phosphorylated OIP30, encoding for a RuvB-like DNA helicase 2 (RuvBL2). Phosphorylation enhances helicase as well as ATPase activity of OIP30, emphasizing it to be downstream substrate of OsPCK25/26 in pollen development (Wang *et al.* 2011). *OsCPK21* predominantly expresses in spikelets, developing seeds, stamens, endosperms, panicles and calli, suggesting its function in reproductive tissues (Ye *et al*. 2009; Asano *et al.* 2011). Functional role of *OsCPK21* in reproductive organ development is yet to be assigned. Another CDPK gene from tobacco, *NtCDPK4* is found to accumulate on stigma surface and young developing anthers (Zhang *et al.* 2005).

Seed

In rice, of the 31 CDPKs, only six (*OsCPK7*, *OsCPK10*, *OsCPK12*, *OsCPK21*, *OsCPK23*, *OsCPK24*) are upregulated during seed development stages, whereas, ten (*OsCPK1*, *OsCPK4*, *OsCPK8*, *OsCPK10*, *OsCPK13*, *OsCPK16*, *OsCPK19*, *OsCPK20*, *OsCPK15* and *OsCPK28*) are downregulated. Except for *OsCPK24*, which is predominately upregulated in the S1 stage of seed development only, the other five genes are also upregulated in panicle development stages (Ray *et al.* 2007). *OsCPK23* (*SPK*), *OsCPK19* (*OsCDPK*2) and *OsCDPK11* (*OsCPK7*) are reported to have role in seed development (Kawasaki *et al.* 1993; Breviario *et al.* 1995; Frattini *et al.* 1999; Morello *et al.* 2000). Rice *SPK* antisense transformants show a defective production of storage starch (Shimada *et al.* 2004). In potato (*Solanum tuberosum* L.), *StCDPK1* transcript accumulates in induced-stolon (IS) but not in vegetative tissue (leaves, shoots, petioles). Moreover, gradual increase in transcript accumulation is observed with progressive development of induced stolon, emphasizing its role in tuberization (Raices *et al.* 2001). Significant transcript accumulation has also been observed on GA, ABA and BA hormone treatment (Gargantini *et al.* 2009). Transgenic lines with reduced expression of *StCDPK1* show earlier tuberization than in control in the absence of GA inhibitor, as well as, more number of tuber than WT even when the WT is treated with hormones that promote tuberization in potato (ABA and BA). Moreover, the transgenics are more insensitive to GA action. Taken together, these findings clearly suggest that StCDPK1 enzyme is involved in GA signaling in developing stolon (Gargantini *et al.* 2009). Another isoform of StCDPK1, StCDPK2, having 86% identity in the catalytic region with StCDPK1, has lower expression in later stages of progressive development of induced stolon (Ullao *et al.* 2002). During the initial developmental stage of induced-stolon (swelling), *StCDPK3* shows transcript accumulation, suggesting a differential role of isoforms of CDPKs in stolon development (Raices *et al.* 2003). A CDPK (*AhCPK1*) gene from peanut (*Arachis hypogaea* L.) expressing in seeds developing under inadequate soil Ca^{2+} condition is also found to be spatiotemporally regulated during early mitotic growth and later, during the storage phase of seed development (Jain *et al.* 2011). It is also established that *AhCPK1* is involved in seed maturation where it has role in maintenance of sink strength and regulation of genes encoding for enzymes which are involved in sucrose cleavage and utilization (Jain *et al.* 2011). The accumulation of another CDPK gene, *FaCDPK1*, from strawberry (*Fragaria* x *ananassa* Duch cv. Pajaro), expressing in roots, stem, stolon, leaves and flowers, also shows gradual increase in transcript accumulation with fruit maturation, suggesting its involvement in fruit development (Llop-Tous *et al.* 2002).

In 1998, Ritchi and Gilroy reported of a 54 kDa CDPK to be involved in GA mediated response of barley (*Hordeum vulgare* L.) aleurone. In a later study, McCubbin *et al*. (2004) cloned two CDPK genes from barley aleurone, *HvCDPK1* and *HvCDPK2*. Similarity in biochemical characters between HvCDPK1 and the earlier isolated 54-kDa protein strongly suggest that they could be same (McCubbin *et al*. 2004). It has also been found that expression of the inactive CDPK form does not alter GA-induced gene expression, but inhibits secretion and vacuolar acidification. Furthermore, application of recombinant HvCDPK1 to isolated aleurone vacuoles results in four-folds increase in ATPase activity and the activity reverses on application of V-ATPase inhibitor, emphasizing on the regulatory role of HvCDPK1 in vacuolar secretion (McCubbin *et al.* 2004).

BIOTIC STRESS

CDPKs have been long associated with decoding of Ca^{2+} signatures during biotic stress response (**Table 1**). In *Arabidopsis*, *AtCPK1* is found to be rapidly induced by fungal elicitors, which was earlier demonstrated to increase NADPH oxidase activity on its ectopic expression (Xing *et al.* 2001). Further, in comparison to WT plants, *cpk1* mutant exhibits higher susceptibility to pathogen infection (Coca and San Segundo 2010). On the other hand, overexpression of *AtCPK1* leads to accumulation of salicylic acid (SA), which in turn regulates downstream expression of SA-regulated defense and disease resistance genes (Coca and San Segundo 2010). On screening for CDPKs involved in flg22 reporter NHL10-LUC, *AtCPK4*, *AtCPK5*, *AtCPK6* and *AtCPK11* emerges as potential early target CDPKs. Further, they are confirmed to be playing key positive roles in initial MAMP signaling (Boudsocq *et al.* 2010). A tomato CDPK gene, *LeCDPK1*, showing transcript accumulation under mechanical wounding, elicitors, polygalacturonide, JA and H_2O_2, not only shows steady state increase at the site of injury but also systemically in distant non-wounded plant parts (Chico *et al.* 2002). *LeCDPK1* is also been found to be involved in cross-tolerance mechanism in tomato. WT tomato plants when subjected to mechanical wounding shows more tolerance to salt stress through generation of systemin (inducer of long-distance wound signal) and JA, which in turn activates downstream signaling cascade having calmodulin-like activities. Next, *LeCDPK1* is also found to be induced on systemin and JA treatment, as well as, high salinity stress. Hence, it is hypothesized that on wounding, JA is generated via octadecanoid pathway triggered by systemin, which in turn induces the expression of *LeCDPK1*, which subsequently phosphorylates downstream components that modulate signaling cascade leading to cross-tolerance (Capiati *et al.* 2006). Another CDPK, NtCDPK1, induced by MJ, SA, fungal elicitor and wounding (Yoon *et al*. 1999; Lee *et al.* 2003), has been found to interact with NtRPN3 regulatory subunit of 26S proteosome and is involved in regulating cell division, differentialtion and cell death. It has also been demonstrated that VIGS silencing of both *NtCDPK1* and *NtRPN3* exhibits HR-like cell death and induction of PR genes, suggesting that the onset of abnormal cellular differentiation and growth triggers the PCD. Two more CDPKs from tobacco, *NtCDPK2* and *NtCDPK3* have also been found to be extensively involved in HR mediated biotic stress response (Romeis *et al.* 2001). Rapid cell-type specific induction under elicitor treatment is noted for *ZmCPK10* (Murillo *et al.* 2001). Another CDPK isolated from maize, *ZmCPK11*, purified from seedlings is activated by phospholipids (Szczegielniak *et al.* 2000) and is involved in wounding generated signaling, but not other stresses. *PaCDPK1* from *Phalaenopsis amabilis* L. is induced in leaf and stem tissue by wounding and pathogen, as revealed by promoter-GUS fusion analysis in *Arabidopsis* (Tsai *et al.* 2007).

Among the rice CDPK genes, *OsCPK7*, *OsCPK9*, *OsCPK13*, *OsCPK15*, *OsCPK17*, *OsCPK20*, *OsCPK23* and *OsCPK24* have been found to respond to fungal elicitors or JA treatment (Kawasaki *et al.* 1993; Akimoto-Tomiyama *et al.* 2003; Asano *et al.* 2005; Wan *et al.* 2007). In wheat, *TaCPK1*, *TaCPK2*, *TaCPK3*, *TaCPK4*, *TaCPK7*, *TaCPK10*, *TaCPK12*, *TaCPK15* and *TaCPK19* responds to powdery mildew infection (Li *et al.* 2008). *TaCDPK1* is upregulated during sucrose treatment to excised wheat leaves, suggesting of this being a part of sucrose induced signaling pathway (Martínez-Noël *et al.* 2007). Role of sugar (i.e. sucrose, trehalose) in maintaining structural integrity of the membranes without interference with cells' normal metabolic process under drought stress condition is well reported (Mahajan and Tuteja 2005). Two CDPK genes isolated from barley, *HvCDPK3* and *HvCDPK4,* on constitutive active expression show compromised penetration resistance to powdery mildew, suggesting negative regulation of CDPK gene in disease resistance (Freymark *et al.* 2007). *CaCDPK3* from *Capsicum anuum* expresses specifically in root tissue under control condition but under osmotic stress and exogenous ABA application, they express in leaves. Transcript accumulation is also observed on treatment of plant defense-related chemicals, ethephon, SA and JA (Chung *et al.* 2004). Potato CDPKs, *StCDPK4* and *StCDPK5*, have been implicated in response to fungal elicitor that results in increased intracellular Ca^{2+} (Kobayashi *et al.* 2007). This Ca^{2+} binds to the StCDPK5 EF-hands and the activated StCDPK5, in turn phosphorylates downstream StRBOHB (Respiratory Burst Oxidase Homolog), resulting in ROS production. Therefore, CDPKs seem to form an integral part of both biotic as well as abiotic stress signal transduction pathways.

ABIOTIC STRESS

CDPKs have been shown to be involved in both biotic and abiotic stress signalling (Ludwig *et al.* 2004). Microarray data show 17 out of 31 CDPKs in rice to be inducible under abiotic stress conditions (Wan *et al.* 2007). Combining this data with extensive northern blot analysis in six rice cultivars, *OsCPK13*, *OsCPK6*, *OsCPK17* and *OsCPK25* are being shown to be important for stress response. *OsCPK6* and *OsCPK25* show gradual upregulation by drought and heat, whereas, *OsCPK17* is downregulated by cold, drought and salt (Wan *et al.* 2007). *OsCPK13* (*OsCDPK7*) is already well characterized to be involved in stress tolerance (Saijo *et al.* 2000). In control 10-day old rice seedlings, *OsCPK13* expression level is very low but exposure to cold and salt stress significantly increases transcription of *OsCPK13* (Saijo *et al.* 2000). Transgenic lines overexpressing *OsCPK13* show enhanced expression of salt and drought stress-induced genes but not cold stress-induced genes. It has been proposed that OsCPK13 acts at one of the branch point of cold and salt/drought responsive pathways, specifically upstream to several late induced genes like *rab16A*, *salT*, *wsi18* (Saijo *et al.* 2000). Working on this hypothesis, *OsCPK13* is expressed in *Sorghum* sp.. However, the transgenic lines are not improved in cold/salt stress, but show a lesion mimic phenotype, implying the induction is more towards biotic response (Mall *et al.* 2011). Even, 2-D gel analysis reveals accumulation of a number of PR-10 proteins due to the transgene effect. Moreover, stimulated accumulation of alanine aminotransferase, NADH-dependent glutamate synthase, acetyl-coenzyme A carboxylase, H^+ ATPase, CIPK23, tonoplast aquaporin, phosphoethanolamine N-methyltransferase and betaine aldehyde dehydrogenase protein is also noted (Mall *et al.* 2011). Hence, the message is very clear that translating phenotypic effects of a transgene in distinct genetic background are not straightforward and ectopic expression could lead to undesirable phenotype. Another CDPK from rice, OsCPK7 (OsCDPK13) protein is found induced by cold stress and GA treatment (Yang *et al.* 2003). When studied in detail, it has been found to be phosphorylated in response to cold stress and GA treatment. Moreover, accumulation of *OsCPK7* transcript is higher in cold-tolerant cultivars than in cold-sensitive varieties (Abbasi *et al.* 2004). Antisense rice transgenic lines are dwarf than WT plants. On the other hand, overexpressing lines are tolerant to cold stress (Abbasi *et al.* 2004). Proteomic analysis of lines overexpressing *OsCPK7* and its related proteins, calreticulin and CRTintP1 (calretuculin interacting protein 1) identifies fructokinase, cytoplasmic malate dehydrogenase and alpha-tubulin to be upregulated (Komatsu *et al.* 2007). It has been suggested that these proteins might be involved in sugar sensing pathway and damage repair caused by cold tolerance and low-temperature stress sensor, respectively. Among the identified wheat CDPK gene family members, 12 CDPK genes have also been implicated in abiotic stress response (Li *et al.* 2008). *IiCPK2*, isolated from *Isatis indigotica* L., is differentially expressed under salinity and cold stress condition as well as on GA treatment. It also has enhanced expression in leaf, root and stem in tetraploid sample than that in diploid progenitor (Lu *et al.* 2006). EST library of *Ulva compressa* cultivated with 10 μM copper for 3 days contains CDPK gene, implying its involvement in copper acclimation and tolerance (Contreras-Porcia *et al.* 2011). Screening of two drought-tolerant barley genotypes ('Martin' and '*Hordeum spontaneum* 41-1 (HS41-1)'), and one drought-sensitive genotype ('Moroc9-75') at the transcriptional level during the reproductive stage under drought conditions reveals that a CDPK gene constitutively expresses in drought-tolerant genotypes, emphasizing on its involvement in drought tolerance (Guo *et al.* 2009). *PgCDPK1c*, *PgCDPK2c* and *PgCDPK4a* isolated from *Panax ginseng* show transcript accumulation in salt stress treated cells, whereas expression of *PgCDPK1b* and *PgCDPK3a* decreases. Expression of *PgCDPK1c*, *PgCDPK2c*, and *PgCDPK4a* are also found enhanced in salt-tolerant *rolB* and *rolC* transformed cell cultures of *P. ginseng* (Kiselev *et al.* 2009).

Another well-characterized CDPK from ice plant, *McCPK1*, exhibits transient increase in transcript and protein accumulation under salt or drought stress. McCPK1 shows a dynamic subcellular localization pattern when it is localized to the plasma membrane in unstressed plants and translocated to nucleus under NaCl stress (Patharkar and Cushman 2000). Even on exposure to low humidity, it relocates extensively to the nucleus, endoplasmic reticulum, and actin microfilaments of the cytoskeleton (Chehab *et al.* 2004). Yeast-two hybrid analysis reveals McCSP1 (two-component pseudoresponse regulator class of transcription factor; Patharkar and Cushman 2000), McCAP1 (*M. crystallinum* CPK1 Adapter Protein 1 having a coiled coil structure; Patharkar and Cushman 2006) and McCAP2 (*M. crystallinum* CPK1 Adaptor Protein 2; Chehab *et al.* 2007) as interacting proteins of McCPK1. Functional physical interaction between McCPK1 and CSP1 proteins are confirmed since McCPK1 is found to phosphorylate McCSP1 in a calcium-dependent manner. Under salt stress condition, McCDPK1 and McCSP1 co-localize in nucleus. However, in control condition, when McCPK1 remains associated with the plasma membrane, McCSP1 exclusively localizes to the nucleus (Patharkar and Cushman 2000). Also, McCAP1 proves to be a poor substrate than McCSP1 for McCPK1. Further, McCPK1 and McCAP1 co-localize in nucleus and cytoplasmic strands of plants on exposure to low humidity condition. Taken together, McCAP1 might be anchoring McCPK1 to cytoskeleton at the time of stress condition (Patharkar and Cushman 2006). On the other hand, McCAP2 is not phosphorylated by McCPK1 but co-localizes with McCPK1 in vesicular and actin microfilament structures as well as ER under low humidity (40%) condition, whereas, under high relative humidity (80%), McCPK1 localizes to the plasma membrane, and McCAP2 remains to vesicle-like structures. McCAP2 also co-localizes with AtVTI1a, a v-SNARE protein known to localize to the *trans*-Golgi network (TGN) and prevacuolar compartments (PVCs; Zheng *et al.* 1999). Hence, it is quite evident that McCPK1 does not phosphorylate McCAP2 but

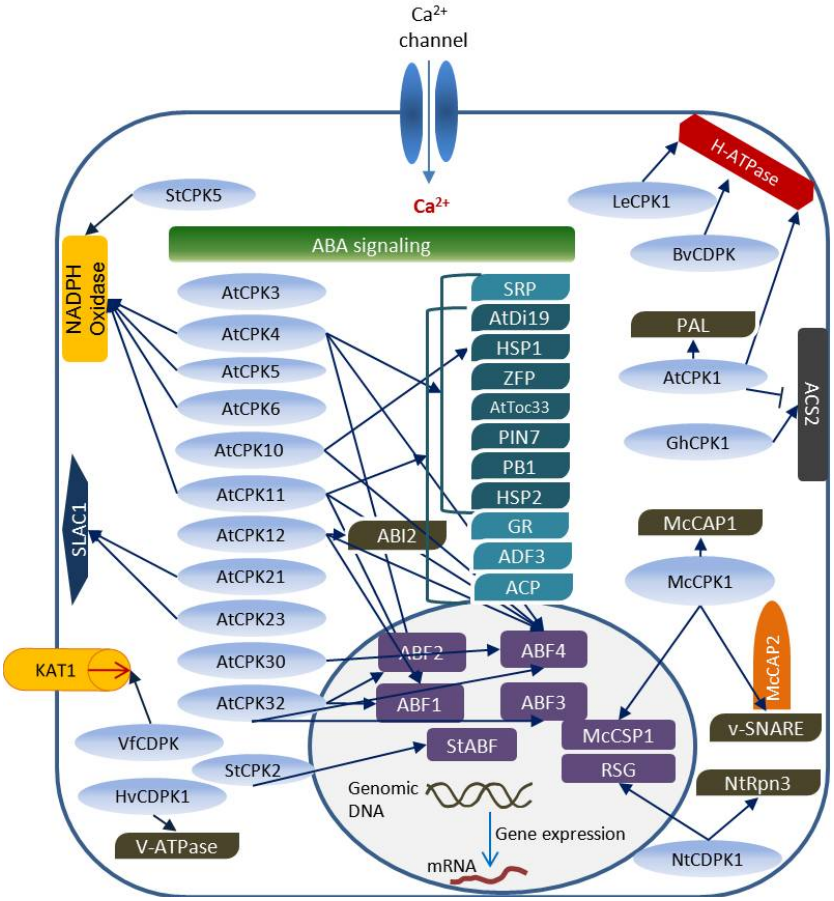

Fig. 2 A model of CDPK targets in plant cell. This model is not exhaustive and examples across plant taxa have been included. Calcium-dependent protein kinase (CDPK/CPK) phosphorylates and interacts with target proteins at different subcellular locations. CDPKs responsive to ABA signaling are discussed under "ABA signaling" tag. Species abbreviation is mentioned as prefix to the protein (i.e. AtCPK1: *Arabidopsis thaliana* calcium-dependent protein kinase 1). Abbreviations: ABF, ABRE binding factor; ABI2, ABA insensitive 2; ACP, Acly-carrier protein; ACS, 1-Aminocyclopropane-1-carboxylic acid synthase; ADF3, Actin depolarizing factor 3; CAP1, CDPK adapter protein 1; CSP1, CDPK substrate protein 1; GR, Geranylgeranyl reductase; HSP1, Heat shok protein 1; KAT1, Potassium channel in *Arabidopsis thaliana* 1; PAL, Phenyl alanine lyase; PB1, Phox and Bem1 domain protein PIN7, Pin-formed 7; Rpn3, Regulatory subunit of 26S proteosome; RSG, Repression of shoot growth; SLAC1, Slow anion channel associated 1; SRP, Serine-rice protein; Toc33, Translocon at the outer envelope membrane of chloroplast protein; ZFP, Zinc finger protein.

it might be possibly serving as an adaptor protein in the protein complex facilitating vesicle transport (Chehab *et al.* 2007). Taken together, McCPK1 may be involved in adaptation mechanism of ice plant to changing relative humidity as well as high salinity, maintaining its water balance all along.

ABA signaling

A significant cross-talk is reported under cold, drought and salinity stress, where ABA is found to be regulating downstream processes to maintain cellular homeostasis. Two main attributes of ABA, include promotion of seed dormancy and avoidance of unfavorable condition along with regulation of stomatal closure to minimize water loss under drought. Under stress condition, both the ABA-dependent signaling pathways co-exist with ABA-independent signaling pathway, where significant cross-talk also exists between these two cascades (Yamaguchi-Shinozaki and Shinozaki 2006). Several CDPKs are found to regulate ABA signal transduction pathways (**Fig. 2**; Choi *et al.* 2005; Mori *et al.* 2006; Zhu *et al.* 2007). *AtCPK10* and *AtCPK30* are activated in response to ABA in protoplast transient expression system (Sheen 1996). AtCPK32 phosphorylates ABF4, a transcriptional regulator in ABA-dependent signaling cascade, at Ser-110 position. In yeast-two hybrid experiment, AtCPK32 is found to interact with ABF4 at its C2-C3 conserved region. *AtCPK32* on overexpression in *Arabidopsis*, confers hypersensitivity to high concentration of ABA and salt during germination. Overexpression also results in induction of ABA-responsive genes (*rd29A*, *rab18* and *rd29*) as well as ABF4-regulated genes. It is not only found that AtCPK32 interacts with more members of ABF family (i.e., ABF1, ABF2 and ABF3) but also ABF4 is found to interact with multiple members of CDPK gene family (AtCPK10 and AtCPK30), emphasizing on their cross-talk during ABA-dependent signaling (Choi *et al.* 2005). *AtCPK4* and *AtCPK11* are found to be induced by ABA application. Their loss-of-function mutant (*cpk4* and *cpk11*) and double mutant (*cpk4cpk11*) show ABA-insensitive phenotypes during seed germination, seedling growth and stomatal movement, which lead to decrease in salt stress tolerance in seedlings (Zhu *et al.* 2007). Conversely, lines overexpressing *AtCPK4* and *AtCPK11* are more sensitive to ABA during seedling growth and stomatal movement. Mutant lines loose more water than overexpression lines during dehydration. Even, total length of lateral roots increases in mutants and decreases in overexpressing lines. The expression of ABA-responsive genes *ABF1*, *ABF2*, *AGF4*, *ABI4*, *ABI5*, *RD29A*, *RAB18*, *KIN1*, *KIN2* and *ERD10* are downregulated in mutant lines and upregulated in overexpression lines (Zhu *et al.* 2007). As these two CPK genes are found to be localized in cytoplasm and nucleus (Dammann *et al.* 2003), it is more likely that they will interact with nuclear-localized transcription factors for delayed ABA-response as well as early response, by phosphorylating downstream messengers in cytosol (Zhu *et al.* 2007). Association of ABA in stimulating cytosolic concentration of Ca^{2+}, which leads to stomatal closure, is well documented (Song *et al.* 2008; Kim *et al.* 2011). Hence, existence of Ca^{2+} sensing signal transducers is obvious in regulation of stomatal conductance (Hubbard *et al.* 2011).

AtCPK3 and *AtCPK6* have been isolated from guard cell-enriched cDNA library and found to express in guard cell as well as mesophyll cells (Kwak *et al.* 2002). ABA-induced stomatal closure and Ca^{2+} reactive stomatal closure are partially impaired in *cpk3cpk6* double mutant plants, whereas, long term Ca^{2+} programmed stomatal closure is not. This differential regulation of R (rapid)- and S (slow)-type anion channels are part of the parallel signal transduction mechanism found in the branched guard cell (Mori *et al.* 2006). MJ and ABA signaling have partial overlap in regulation of guard cell signaling (Munemasa *et al.* 2007; Saito *et al.* 2008). *AtCPK3*, *AtCPK6*, *AtCPK4* and *AtCPK11* disruption mutants have been screened for stomatal phenotype and found that MJ activation of I_{Ca} channels and S-type anion channels are disrupted in *AtCPK6* disruption (*cpk6*) mutants and it functions as a positive regulator of MJ signaling in *Arabidopsis* guard cells by a feedback loop (Munemasa *et al.* 2011). In a recent study, *AtCPK10* mutant (*cpk10*) has been found to be sensitive to drought and complemented lines showed recovery of phenotype (Zou *et al.* 2010). HSP1 (Heat Shock Protein 1) has been identified as AtCPK10-interacting protein. Moreover, *hsp1* mutants also show similar effects on plant response to drought stress, as seen in *cpk10* mutant. Importantly, ABA and Ca^{2+} mediated inhibition of the inward K^+ currents is impaired in both the *cpk10* and *hsp1* mutants. Taken together, it has been demonstrated that AtCPK10 and HSP1 function in the regulation of stomatal movements via ABA and Ca^{2+} signaling pathways during drought stress (Zou *et al.* 2010). In an earlier report, *AtCPK10* and *AtCPK11* have been reported to be inducible under cold, salinity and drought stress by Urao *et al.* (1994). To understand SLAC1-mediated ABA signaling cascade, *SLAC1* has been expressed together with *AtCPK3*, *AtCPK6*, *AtCPK21*, *AtCPK23*, *AtCPK31*, *ABI1* and *HAB1* in *X. laevis* oocytes and the interaction is visualized with the bimolecular fluorescence complementation technique (BiFC). It was deciphered that on reception of ABA in stomata, ABI1 is inactivated by the ABA-receptors. This relieves inhibition of AtOST1, AtCPK23 and AtCPK21, and finally SLAC1, a guard cell anion channel, is activated by phosphorylation resulting in concomitant water loss from guard cells and closure of stomatal pore (Geiger *et al.* 2010). In an earlier report, *cpk23* mutant showed enhanced tolerance to drought and salt stresses, while the *AtCPK23* overexpression lines were more sensitive to drought and salt stresses, and the complementary lines displayed recovery of phenotype comparable to WT plants (Ma and Wu 2007). This phenomenon has been explained by reduced stomatal aperture in mutant lines. It has also been concluded that AtCPK23 mediated salt tolerance by regulating K^+-uptake (Ma and Wu 2007). K^+ channel, KAT1 has also been reported to be phosphorylated by CDPK from *Vicia faba* guard cells (Li *et al.* 1998). There is also evidence of negative regulation of ABA by CDPK. During seed germination and post-germination growth, AtCPK12 is involved in negative ABA-signaling. AtCPK12 interacts and phosphorylates a protein phosphatase, ABI2, and also phosphorylates ABF1 and ABF4 *in vitro*. Thus, it is seen that CDPKs modulate ABA signaling in a loop regulation (Zhao *et al.* 2011). ABA mediated signaling of CDPK genes is also demonstrated in other plant species. *ACPK1*, expressed in the mesocarp of grape berries, is found to be stimulated by ABA in a dose-dependent manner. Moreover, alteration in expression and activity of ACPK1 occurs in a synchronized manner with the endogenous ABA concentrations during fruit development. Further it has been concluded that ACPK1 may be positively involved in ABA-signaling pathway, and promoted plasmalemma H1-ATPase-powered active in grape berry (Yu *et al.* 2006). Heterologous overexpression of *ACPK1* in *Arabidopsis* elevates plant biomass production as well as increased ABA-sensitivity in seed germination, early seedling growth and stomatal movement; hence, they conclude that ACPK1 is a positive regulator in ABA signal transduction (Yu *et al.* 2007). Another orthologous CDPK protein to OsCPK2 and AtCPK9, is isolated from *Beta vulgaris* root that phosphorylates the H^+-ATPase in a calcium-dependent manner (Lino *et al.* 2006). *VfCPK1* isolated from epidermal peels of broad bean (*Vicia faba* L.) leaves shows differential accumulation of mRNA and protein in leaves treated with ABA and drought stress, which clearly indicates that this enzyme might be regulating ABA-dependent drought signaling in epidermal cells (Liu *et al.* 2006). *CaCDPK3* is also found to be induced on exogenous ABA treatment (Chung *et al.* 2004). It is quite clear from the above-discussed studies that CDPKs across species are intricately involved in ABA-mediated stress signaling pathway.

SUBCELLULAR LOCALIZATION: INTERACTION WITH THE TARGET

Functionality of a protein depends a lot on its subcellular localization, as access and availability of interacting partners are determinants for transduction of signals. Targeting sequences, mostly in the N-terminal domain of the protein as well as general properties like hydrophobicity of proteins determine the location (Sachs and Engelman 2006). Membrane association of proteins is mainly due to the presence of hydrophobic transmembrane domain, electrostatic interaction with membrane components and lipid modifications (Sachs and Engelman 2006). The highly variable N-terminal domain present in CDPKs contains information regarding subcellular targeting namely, N-myristoylation and palmitoylation sites (Cheng *et al.* 2002). N-myristoylation and palmitoylation signal is abundantly found in proteins involved in signal transduction (Taniguchi 1999; Iwanaga *et al.* 2009). N-myristoylation is post-translational attachment of myristic acid to the glycine at the second position of the N-terminal domain (Taniguchi 1999). Palmitoylation is the post-translational attachment of palmitic acid to cysteine residue, which could be positioned in the N-terminal domain or internal position (Iwanaga *et al.* 2009). Myristoylation facilitates membrane binding of proteins in a reversible manner. However, anchoring to membrane becomes stable if palmitoylation event follows myristoylation (Taniguchi 1999). Out of 34 CDPKs from *Arabidopsis*, 24 have been predicted to contain myristoylation site (Cheng *et al.* 2002). Similarly, in rice, out of 31 CDPKs, 18 proteins bear this site (Asano *et al.* 2005; Ray *et al.* 2007). In case of wheat CDPKs, full-length clone data is not available for all, hence, of the 14 CDPKs for whom the N-terminal data is available, only three isoforms are predicted to have myristoylation site (Li *et al.* 2008). In agreement to the structural diversity, CDPKs are located in cytosol (Dammann *et al.* 2003; Ray *et al*. unpublished data), plasma membrane (Yoon *et al.* 1999; Damman *et al.* 2003), endoplasmic reticulum (ER) membrane (Lu and Hrabak 2002), peroxisome (Damman *et al.* 2003), cytoskeleton (Putnam-Evans *et al.* 1989), endosperm storage vesicles (Anil *et al.* 2003), mitochondria (Pical *et al.* 1993) and nucleus (Patharkar and Cushman 2000; Damman *et al.* 2003). When subcellular localization of eight *Arabidopsis* CDPKS (AtCPK1, AtCPK3, AtCPK4, AtCPK7, AtCPK8, AtCPK9, AtCPK16, AtCPK21 and AtCPK28) were studied by generating transgenic lines expressing AtCPK-GFP fusion construct, AtCPK3 and AtCPK4 localized in nucleus as well as cytosol, suggesting them to be soluble proteins with potential to target nucleus, where, six CDPKs (AtCPK7, AtCPK8, AtCPK9, AtCPK16, AtCPK21 and AtCPK28) localized in nuclear membrane (Damman *et al.* 2003). Interestingly, AtCPK1 localized with peroxisomal bodies (Damman *et al.* 2003). In a later study, it has been observed that *AtCPK1* regulates plant innate immunity via SA-dependent signaling pathway and showed dual localization in lipid bodies and peroxisomes. Association of lipid bodies with disease is well documented in mammalian cells and in this study, several Toll-interleukin receptors are also found to be regulated by AtCPK1. Moreover, lipid bodies containing AtCPK1 are located near peroxisomes. This close association of oil bodies and peroxisomes facilitates transfer of

fatty acids from the lipid bodies to peroxisomes and is used for peroxisomal fatty acid β-oxidation. Hence, localization of AtCPK1 in oil bodies and its role in defense response establishes link between plant immunity and protein compartmentalization (Coca and San Segundo 2010). In 2008, Benetka *et al*. reported that AtCPK2 localizes on plasma membrane and some distinct spots in the cytosol (not nucleus), AtCPK6 localizes on membrane and nucleus, whereas, AtCPK9 and AtCPK13 localize precisely on plasma membrane. Moreover, disruption in myristoylation site results in loss of target location and reallocation of these proteins mainly to cytosol (Benetka *et al.* 2008). In another article, determinant role of myristoylation and palmitoylation in cellular localization has also been well demonstrated for AtCPK16. AtCPK16 native protein localizes predominantly at the plasma membrane. On replacing glycine on position 2 with alanine, which abolishes myristoylation but not palmotylation, AtCPK16 locates to chloroplasts. Conversely, the mutant protein, which can be myristoylated but not palmitoylated, does not localize with chloroplast but to nucleus. The double mutant with impaired myristoylation and palmitoylation again localizes in chloroplasts, thus suggesting that myristoylation alone inhibits chloroplast localization of CPK16 and presence of both the signals determine membrane localization (Stael *et al.* 2011). In a series of reports over time, AtCPK4, AtCPK5, AtCPK6 and AtCPK11 have been found to have dual functionalities and their subcellular localization seem to facilitate the same. AtCPK11 locates in cytoplasm and nucleus of *Arabidopsis* protoplast while, its interacting partner, AtDi19, a zinc-finger protein, locates in nucleus. Hence, *in vitro* interaction of them is consistent with their localization pattern (Milla *et al.* 2006a). Involvement of this CDPK has been established well as AtDi19-related genes are also reported to be stimulated by drought and salt stresses (Milla *et al.* 2006b). Moreover, AtCPK11 and AtCPK4 phosphorylate transcription factors ABF1 and ABF4 and mediate ABA signaling as well as regulate a number of stress tolerance related genes (Zhu *et al.* 2007). Furthermore, AtCPK4, AtCPK11, AtCPK5 and AtCPK6 have role in innate immunity and have been shown to regulate ROS production, by directly phosphorylating the NADPH oxidase RBOHB as well as modulate early target genes which are also regulated by flg22 within 30 to 60 min in mesophyll protoplasts, seedlings and leaves (Boudsocq *et al.* 2010). Therefore, dual location of AtCPK4, AtCPK5, AtCPK6 and AtCPK11 could be mediating a quick response by sensing Ca^{2+} and phosphorylating downstream players, while the nuclear localized ones would phosphorylate transcription factors that mediate gene expression. Similarly, AtCPK32 has been found to interact with ABF4 and localized in the nucleus (Choi *et al.* 2005). AtCPK21 and AtCPK23 are involved in drought response (Geiger *et al.* 2010; Franz *et al.* 2011) via ABI mediated regulation of guard cell anion channel SLAC1. Again, membrane localization of both the CPKs is in tune with their function (Geiger *et al.* 2010). A membrane bound CDPK from rice was reported as early as in 1993 by Morello *et al*. Two years later, another GA-inducible CDPK was isolated from seed, which was also membrane bound (Abo-el Saad and Wu 1995). OsCPK4 and OsCPK18 are located in plasma membrane and on mutation of the myristoylation site, relocate to cytoplasm. These CPKs are induced by AM fungus *G. intraradices*, hence their membrane location might be helpful in perception of the fungal-produced symbiotic signal (Campos-Soriano *et al.* 2011). Another CDPK, OsCPK2 have been found to be membrane localized as well as requirement of both myristoylation and palmitoylation site for the purpose have been demonstrated well (Martin and Busconi 2000). Martin and Busconi in (2001) identified another low temperature inducible membrane localized rice CDPK protein. Cytoplasm localized CDPKs are also reported from rice. On expressing OsCPK24:GFP fusion protein in tobacco protoplast, OsCPK24 localizes exclusively in cytoplasm (Zhang *et al.* 2005). OsCPK21, involved in ABA response pathway as well as salt tolerance on overexpression (Asano *et al.* 2011), is localized in nucleus (Ray *et al.* unpublished data). Dual localization of CDPKs has also been reported in rice where, OsCPK13 localizes in cytoplasm and nucleus and its functionally confers salt/drought tolerance, but till the interacting proteins and signaling pathways are identified for this enzyme, the relevance of subcellular localization remains unclear. However, earlier reports reflected that dual localization (cytoplasm and nucleus) of AtCPK4, AtCPK5, AtCPK6 and AtCPK11 could be facilitating their dual functionality mode (Zhu *et al.* 2007; Boudsocq *et al.* 2010). Similar facts could be true for rice CDPKs having dual location in cells which needs to be worked out in the future.

Other than rice and *Arabidopsis*, CDPKs from other plant species have also been found to be located in diverse subcellular locations. Native StCDPK1, LeCPK1 and NtCPK5 are membrane bound. However, mutated myristoylation and palmotylation site target protein to cytoplasm (Rutschmann *et al.* 2002; Raices *et al.* 2003; Wang *et al.* 2005). CaCDPK3 localizes to the cytosol in chili pepper protoplasts (Chung *et al.* 2004). AhCPK2 from peanut is located in both membrane and soluble fractions under normal and stressed condition, respectively. However, under normal condition it does not localize in the nuclear fraction, but is clearly detectable in the nuclear fraction of the cells when subjected to 0.4 M sucrose for 4 days. It has also been demonstrated that this differential nuclear localization is specific to stress response, since following auxin treatment to peanut cells, the subcellular localization remains unchanged (Raichaudhuri *et al.* 2006). On the same note, McCPK1 also shows stress dependent differential localization in cellular compartments (Patharkar and Cushman 2000; Chehab *et al.* 2004). Both isoforms, CaCPK1 and CaCPK2 from chickpea are located in the plasma membrane and chloroplast membrane of leaf mesophyll cells, as well as in the membrane of stem xylem parenchyma cells (Syam and Chelliah 2006a). PiCDPK1 localizes preferably at the periphery of the pollen tube consistent with a plasma membrane location, unlike PiCDPK2, which localizes to internal membrane compartments (Yoon *et al.* 2006). Truncated PiCDPK1 (ΔN-PiCDPK1), on losing its target signal, localizes in the cytosol rather than the plasma membrane and interestingly, results in normal pollen tube growth in contrast to the loss of polarization in pollen tubes, which is exhibited on full-length protein expression. These findings showcase that assessment of subcellular localization of proteins is important parameter for determining the cellular functionality and their role in signaling cascade.

CDPKs IN SIGNALING CROSS-TALK

CDPKs have been documented to be involved in developmental process, biotic and abiotic stress signaling pathways, although little is known about their participation in cross-talk between these pathways. However, a major challenge in understanding the role of individual CDPK is the presence of multiple isoforms, which have very specific inducibility in specialized cell types as well as wide substrate base (Ludwig *et al.* 2004). The cross-talk networking of CDPK genes is represented in **Fig. 2**.

In monocot species rice, *OsCPK7* confers cold stress tolerance via sugar sensing pathway, involve in seed development and is induced by GA but suppressed in response to ABA and brassinolide. Hence this CPK enzyme must be the convergence point among these distinct pathways (Kawasaki *et al.* 1993; Abbasi *et al.* 2004; Komatsu *et al.* 2007). In OsCPK13 overexpressing transgenics, salt and drought responsive target genes are upregulated but not of cold stress, thus indicating that OsCPK13 may be acting at a junction point between two distinct pathways of cold and salt/drought stress tolerance (Saijo *et al.* 2000). Reports reveal that *OsCPK19* and *CsCDPK5* are regulated by seed development and phytohormones signaling pathway, respectively as well as light (Morello *et al.* 2000; Kumar *et al.* 2004). StCDPK2 expression has also been found to be

regulated by light (Giammaria *et al.* 2011). Moreover, it is also found to phosphorylate StABF in a tuber-inducing condition but inhibits in presence of GA (Muñiz García *et al.* 2011). Strikingly, *StABF* is induced in response to ABA, drought, salt stress and cold stress, suggesting involvement of *StCDPK2* in multiple signaling cascades. Cross-talk between multiple hormone and stress conditions has also been seen for *NtCDPK1*, where it is induced by wounding as well as by phytohormone treatment, high salt and by fungal elicitor. NtCDPK1 regulates endogenous GA content via RSG (Fukazawa *et al.* 2000; Igarashi *et al.* 2001; Ishida *et al.* 2004, 2008). RSG regulates GA biosynthesis via *ent*-kaurene oxidase (*NtKO*) and GA 20-oxidase (*NtGA20ox1*) genes (Fukazawa *et al.* 2010) where the expression of *NtGA20ox1* gene is regulated by binding of RSG to its promoter, stimulated by a decrease in GA levels. Moreover, active histone marks are also modified in the promoter region by decrease in GA levels (Fukazawa *et al.* 2010, 2011). Additionally, another gene, *NtCPK4* is also differentially regulated under salt stress condition and GA treatment (Zhang *et al.* 2005). *ZmCPK11* have been found to be component of touch- and wound-induced pathway(s) and involved in early stages of local and systemic responses. Moreover, it is also suggested to be having role in post-germination growth (Szczegielniak *et al.* 2000, 2005, 2012). *PaCDPK1* expresses differentially in labellum and peloric flower. Also, its promoter is found to be induced by abiotic stress (Tsai *et al.* 2007). Another example of cross-talk is *AtCPK32*, which is responsive to signaling hormone ABA (Choi *et al.* 2005), induced by touch, wounding, NaCl and darkness but little or no response to other hormones like ethylene and MJ (Chotikacharoensuk *et al.* 2006), hinting AtCPK32 to be at the converging point of diverse stress responses.

The already complex signaling network turns out to be much more complicated when Uno *et al.* (2009) identified the interacting partners of AtCPK4 and AtCPK11 (share 95% similarity) by high-throughput yeast-two hybrid interaction. AtCPK4 has been found to interact with 14 redundant proteins and 16 proteins have given single hit. The five most redundant interacting proteins are AtDi19, HSP1, serine-rich protein, zinc-finger protein and AtToc33. On the other hand, AtCPK11 interacts with 24 different proteins, in which 13 are redundant preys. Moreover, of the redundant ones, both the CDPKs interact with AtDi19, HSP1, HSP2, zinc-finger protein, AtToc33, Pin7, PB1 domain-containing protein. Hence, to highlight the above, AtCPK4 and AtCPK11 interact with proteins involved in wide cellular processes like, hormone signaling (Pin7), translocation of nuclear encoded pre-protein into chloroplast (AtToc33), stress-response factors (HSP1, HSP2, AtDi19). Strikingly, even after having 95% protein identity they even show specific interactions suggesting that CDPK have precise post translational regulation. Even these interacting proteins are localized in different compartments in the cell like chloroplast, nucleus (Uno *et al.* 2009). In conclusion, CDPKs percept wide range of signals and integrate at different levels of signaling cascades modulating the appropriate downstream responses. Only further detailed experimental evidence can reveal the nodal points of convergence during signaling.

CDPK and MAPK cross-talk

Cross-talk between Ca^{2+}-mediated MAP kinase is well understood in animal systems, where calmodulins are known to regulate MAPK pathway (Agell *et al.* 2002). In plant cells, both CDPKs and MAPKs have already been identified to be part of plant immunity signaling (Romeis *et al.* 2001). On perception of various environmental cues, CDPK along with MAPK constitute two important signaling cascades operating to regulate the downstream cellular processes for stress response. *NtCDPK2* and *NtCDPK3*, falling in the same subfamily, are differentially induced by wounding and Avr9, respectively. Even on elicitor treatment, steep increase in kinase activity of NtCDPK2 has been observed. On silencing of *NtCDPK2*, necrotic symptoms in Cf-4/Avr4 and Cf-9/Avr9 cell lines are significantly reduced and even leaf-wilting phenotype in CDPK-silenced plants is not observed, but the WIPK accumulation remains unaltered. On the other hand, flooding of CDPK-silenced leaves show flooding-induced activation of MAPKs, WIPK and SIPK, suggesting that NtCDPK subfamily members and MAP kinase are distinct operating pathways (Romeis *et al.* 2001). Further, *NtCDPK2* is found to be involved in to hypoosmotic (abiotic) stress where, NtCDPK2 overexpressing lines show transcript accumulation of HR genes like *HinI*, *topxC1*, *PR1b*, *PR2b* and *PI-II* but not *PR1a* and *PR2a*, which are controlled by SA-regulated pathway. Besides, JA and ethylene show elevated level in *NtCDPK2*-VK expressing leaves, whereas, SA level is unaltered. These findings emphasize on NtCDPK2 being the converging point between biotic and abiotic stress via ethylene and JA (Ludwig *et al.* 2005). Interestingly, contrary to the earlier opinion that MAPK expression is independent of NtCDPK2, inhibition of stimulus-dependent activation of MAPK is evident even on constitutive active expression of NtCDPK2. Furthermore, this inhibition is found to be ethylene mediated, hence confirming an intricate and balanced interplay of stress regulation, mediated by CDPK and MAPK (Ludwig *et al.* 2005). On expressing constitutively active CPK5ac, CPK11ac and MKK4a in *Arabidopsis* protoplast, *FRK1* (*flg22-Induced Receptor Kinase 1*) is found to be MAPK-specific, whereas, *PHI* (*Phosphate Induced 1*) is CDPK specific. *NHL10*, *PER62* (*Peroxidase 62*) and *PER4* are induced by both CDPK and MAPK. However, Ca^{2+} blockers cannot abolish flg22-mediated activation of *MAPK* or *NHL10*. Again, CPKacs does not activate MPK3 or MPK6. Double (*cpk5 cpk6)* and triple (*cpk5 cpk6 cpk11*) mutants by genetic crosses and quadruple mutant (*cpk4 cpk5 cpk6 cpk11*) is generated by virus-induced gene silencing (VIGS). Although, *cpk5 cpk6* and *cpk5 cpk6 cpk11* show loss of activation of 60-kDa CDPKs on flg22 activation, MAPK is still activated by flg22 in these mutant lines. Even flg22-CDPK-specific target genes show differential expression in mutant lines, whereas, expression of FRK1 is not altered. However, CYP81F2, WAK2 and FOX activation is equally dependent on MAPK and CDPK, where activity of the former is uniquely dependent on AtCPK5/AtCPK6 and MAPK. These findings clearly explain the cross-talk between MAPK and CDPK as well as independent regulatory cascade independent of each other (Boudsocq *et al.* 2010).

StCDPK5 has been found to be involved in ROS signaling along with MAPK pathway. On recognition of pathogen signal, the initial transient influx of Ca^{2+} into cytoplasm triggers ROS burst and onset of HR response (Grant *et al.* 2000). StCDPK5, localized to membrane, induces phosphorylation of RBOHs and regulates ROS burst but not NO (nitric oxide; Kobayashi *et al.* 2007). Concomitantly, StMEK2 on transient expression induces ROS production and HR-like cell death (Katou *et al.* 2003, 2005). MEK2 also activates SIPK, which in turn upregulates inducible form of RBOH and ROS as well as NOA1-mediated NO production (reviewed by Yoshioka *et al.* 2011). Hence, here we notice that CDPK and MAPK are parallel pathways promoting ROS production on advent of biotic stress but are interlinked.

EVOLUTION OF CDPK GENE FAMILY

Arabidopsis codes for 34 CDPK genes, which are distributed among five chromosomes. Depending on sequence homology, they cluster into four distinct groups (Cheng *et al.* 2002; Hrabak *et al.* 2003). Similar attempts by using the rice genome sequence reveals 31 CDPK genes (Asano *et al.* 2005; Ray *et al.* 2007). A phylogenetic analysis of rice and *Arabidopsis* CDPK superfamily reveals seven groups, where CDPKs are distributed in four subgroups. CDPK-related kinases (CRKs), calcium- and calmodulin-depen-

dent protein kinases (CCaMKs) and phosphoenolpyruvate related kinases (PEPRKs) are clustered in three distinct classes. Since rice and *Arabidopsis* CDPKs are represented in all four subgroups; certain level of divergence pre-exists before bifurcation of monocot and dicot. However, there might not have been major evolutionary change in CDPK population after the bifurcation, indicating their involvement in essential cell functions (Ray *et al.* 2007). In wheat, 20 CDPK genes are identified and they cluster in similar four groups as in *Arabidopsis* and rice (Li *et al.* 2008). After monocot and dicot divergence, these families expanded independently as has been seen in case of *Arabidopsis* and rice, where five *Arabidopsis* AtCPK genes (*AtCPK21*, *AtCPK22*, *AtCPK23*, *AtCPK27* and *AtCPK31*) are duplicated in tandem orientation and all nine pairs of CDPKs in rice (*OsCPK1*/*15*, *OsCPK2*/*14*, *OsCPK3*/*16*, *OsCPK5*/*13*, *OsCPK13*/*23*; *OsCPK11*/*17*, *OsCPK21*/*22*, *OsCPK25*/*26* and *OsCPK24*/*28*) result only from segmental duplication event (Cheng *et al.* 2002; Ray *et al.* 2007). The CDPK multigene families have also been characterized in soybean, tobacco, ice plant, maize and *Petunia* (Ludwig *et al.* 2004). CDPK isoforms show homology ranging from 99-20%, and it is still a puzzle if close homologues or orthologues will have functional redundancy. If we look at the expression profile of the duplicated genes in rice, four pairs (*OsCPK1/15*, *OsCPK3/16*, *OsCPK2/14*, *OsCPK5/13*) have retention of expression, three pairs (*OsCPK13/23*, *OsCPK21/22*, *OsCPK24/28*) show neo-functionalization and only one pair (*OsCPK11/17*) results into pseudogenization. It has also been observed that divergence of expression is due to modification in occurrence of *cis*-regulatory elements in promoter region. Ray *et al*. (2007) have performed expression hierarchical clustering of 31 CDPKs in rice with 17 stages of development and found that group 8 comprises of six CDPKs (*OsCPK25/26*, *OsCPK6*, *OsCPK14*, *OsCPK2*, *OsCPK22* and *OsCPK29*) expressing during the P6 stages of panicle development. Continually in 2009, Ye *et al.* have studied expression of CDPKs in 27 stages of rice development and found *OsCPK2*, *OsCPK22*, *OsCPK29* and *OsCPK25*/26 to have stamen and panicle preferential expression. Interesting fact is that *OsCPK2*, *OsCPK14*, *OsCPK25*, *OsCPK26* (Group-IIa) and *OsCPK22*, *OsCPK29* (Group-IIIa) are also structurally related (Ray *et al.* 2007). Rice CDPKs, *OsCPK18*, *OsCPK4*, *OsCPK30* and *OsCPK31*, cluster in Group IV, which appears to have diverged significantly from the other rice CDPK sequences. Moreover, they seem to be more related to CCaMKs. Markedly, *OsCPK18* and *OsCPK4* are both upregulated by the AM fungus, *G. intraradices*, correlating structural relatedness to functional relatedness (Campos-Soriano *et al.* 2011). However, in *Arabidopsis*, *AtCPK10* and *AtCPK30* belong to subgroup III of CDPKs, and interact with ABF4. Another member from subgroup IV, *AtCPK28*, does not interact with ABF4 (Choi *et al.* 2005). Hence, it is found that we encounter both the case where structural relatedness can be related with functional property and also unrelatedness. Even orthologues always do not mean functional redundancy. In contrary to tobacco *NtCDPK2*, which is known to have critical role in plant defense, its *Arabidopsis* orthologues, CPK1ac and CPK2ac, despite their relatively high kinase activity, does not show significant induction of NHL10-LUC expression (Boudsocq *et al.* 2010).

CONCLUSIONS

Literature shows that CDPKs are intricate part of Ca^{2+} mediated signaling cascade. However, more reports of thorough characterization of this gene family are still required to fully understand the network. Although, regulatory role of the variable N-terminal domain in substrate specificity is evident, there are few reports on post translational regulation mechanism of these genes. Further research in this aspect would give clearer picture of the diverse substrate base of these genes. We already have reports showing CDPKs being present at nodal positions in multiple signaling cascades. So, post-translational regulation enhances the understanding. The presence of multiple isomers of CDPKs in a plant species and their functional redundancy makes it difficult to characterize. Since it is known that CDPKs recognize Ca^{2+} oscillations in cytosol, emphasis on studies to identify these precise Ca^{2+} signatures and their specificities towards isoforms would also be given. The sequence variation in the CLD is speculated to be involved in differential regulation; however, more biochemical studies are required to prove the hypothesis. Duplication events are resulting in expansion of this gene family in plant species. Diversity or relatedness in expression profile of the duplicated gene pairs is also reported. However, comprehensive functional characterization of the pairs is still not being done. This kind of analysis would reveal the functional evolutionary significance of CDPK genes. Recent advances in proteomic tools like TAP-Tagging, protein array and BiFC imaging could accelerate discovery of components involved in the signaling cascade and their cross-talks. Development of single/multiple gene mutants in *Arabidopsis* as well as other important crops like rice would help delineate the signaling network. Ultimately, the task would be to combine our knowledge from biochemical, genetic and functional studies to comprehend a model of Ca^{2+} signaling pathway in relation to whole plant physiology.

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REFERENCES

Abbasi F, Onodera H, Toki S, Tanaka H, Komatsu S (2004) *OsCDPK13*, a calcium-dependent protein kinase gene from rice, is induced by cold and gibberellin in rice leaf sheath. *Plant Molecular Biology* **55**, 541-552

Abo-el Saad M, Wu R (1995) A rice calcium-dependent protein kinase is induced by gibberellin. *Plant Physiology* **108**, 787-793

Agell N, Bachs O, Rocamora N, Villalonga P (2002) Modulation of the Ras/Raf/MEK/ERK pathway by Ca^{2+}, and calmodulin. *Cell Signal* **14**, 649-654

Akimoto-Tomiyama C, Sakata K, Yazaki J, Nakamura K, Fujii F, Shimbo K, Yamamoto K, Sasaki T, Kishimoto N, Kikuchi S, Shibuya N, Minami E (2003) Rice gene expression in response to N-acetylchitooligosaccharide elicitor: Comprehensive analysis by DNA microarray with randomly selected ESTs. *Plant Molecular Biology* **52**, 537-551

Anil VS, Harmon AC, Rao KS (2003) Temporal association of Ca^{2+}-dependent protein kinase with oil bodies during seed development in *Santalum album* L.: Its biochemical characterization and significance. *Plant Cell Physiology* **44 (4)**, 367-376

Anil VS, Rao KS (2001) Purification and characterization of a Ca-dependent protein kinase from sandalwood (*Santalum album* L.): Evidence for Ca^{2+}-induced conformational changes. *Phytochemistry* **58**, 203-212

Asano T, Hakata M, Nakamura H, Aoki N, Komatsu S, Ichikawa H, Hirochika H, Ohsugi R (2011) Functional characterisation of OsCPK21, a calcium-dependent protein kinase that confers salt tolerance in rice. *Plant Molecular Biology* **75 (1-2)**, 179-191

Asano T, Kunieda N, Omura Y, Ibe H, Kawasaki T, Takano M, Sato M, Furuhashi H, Mujin T, Takaiwa F, Wu Cy CY, Tada Y, Satozawa T, Sakamoto M, Shimada H (2002) Rice SPK, a calmodulin-like domain protein kinase, is required for storage product accumulation during seed development: Phosphorylation of sucrose synthase is a possible factor. *Plant Cell* **14 (3)**, 619-628

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 Physiology* **46**, 356-366

Becker JD, Boavida LC, Carneiro J, Haury M, Feijó JA (2003) Transcriptional profiling of *Arabidopsis* tissues reveals the unique characteristics of the pollen transcriptome. *Plant Physiology* **133 (2)**, 713-725

Benetka W, Mehlmer N, Maurer-stroh S, Sammer M, Koranda M, Betschinger J, Knoblich JA, Teige M, Eisenhaber F (2008) Experimental testing of predicted myristoylation targets involved in asymmetric cell division and calcium-dependent signaling. *Cell Cycle* **7 (23)**, 3709-3719

Berberich T, Kusano T (1997) Cycloheximide induces a subset of low temperature-inducible genes in maize. *Molecular and General Genetics* **254**, 275-283

Botella JR, Arteca JM, Somodevilla M, Arteca RN (1996) Calcium-dependent protein kinase gene expression in response to physical and chemical stimuli in mungbean (*Vigna radiata*). *Plant Molecular Biology* **30**, 1129-1137

Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng S, Sheen J (2010) Differential innate immune signalling via Ca^{2+} sensor protein kinases. *Nature* **464 (7287)**, 418-422

Breviario D, Morello L, Giani S (1995) Molecular cloning of two novel rice cDNA sequences encoding putative calcium-dependent protein kinases. *Plant Molecular Biology* **27 (5)**, 953-967

Bulgakov VP, Gorpenchenko TY, Shkryl YN, Veremeichik GN, Mischenko NP, Avramenko TV, Fedoreyev SA, Zhuravlev YN (2011) CDPK-driven changes in the intracellular ROS level and plant secondary metabolism. *Bioengineered Bugs* **2 (6)**, 327-330

Campos-Soriano L, Gómez-Ariza J, Bonfante P, San Segundo B (2011) A rice calcium-dependent protein kinase is expressed in cortical root cells during the presymbiotic phase of the arbuscular mycorrhizal symbiosis. *BMC Plant Biology* **19**, 11-90

Capiati DA, País SM, Téllez-Iñón MT (2006) Wounding increases salt tolerance in tomato plants: evidence on the participation of calmodulin-like activities in cross-tolerance signalling. *Journal of Experimental Botany* **57 (10)**, 2391-2400

Chang W, Fu G, Chen X, Zhu J, Zhang Z (2011) Biochemical characterization of a calcium-sensitive protein kinase LeCPK2 from tomato. *Indian Journal of Biochemistry and Biophysics* **48 (3)**, 148-153

Chang WJ, Su HS, Li WJ, Zhang ZL (2009) Expression profiling of a novel calcium-dependent protein kinase gene, *LeCPK2*, from tomato (*Solanum lycopersicum*) under heat and pathogen-related hormones. *Bioscience, Biotechnology and Biochemistry* **73 (11)**, 2427-2431

Chehab EW, Patharkar OR, Cushman JC (2007) Isolation and characterization of a novel v-SNARE family protein that interacts with a calcium-dependent protein kinase from the common ice plant, *Mesembryanthemum crystallinum*. *Planta* **225 (4)**, 783-799

Chehab EW, Patharkar OR, Hegeman AD, Taybi T, Cushman JC (2004) Autophosphorylation and subcellular localization dynamics of a salt- and water deficit-induced calcium-dependent protein kinase from ice plant. *Plant Physiology* **135 (3)**, 1430-1446

Cheng S, Willmann MR, Chen H, Sheen J (2002) Update on calcium signaling calcium signaling through protein kinases. The *Arabidopsis* calcium-dependent protein kinase gene family. *Plant Physiology* **129**, 469-485

Chico MJ, Raíces M, Téllez-Iñón TM, Ullao MR (2002) A calcium-dependent protein kinase is systemically induced upon wounding in tomato plants. *Plant Physiology* **128**, 256-270

Choi HI, Park HJ, Park JH, Kim S, Im MY, Seo HH, Kim YW, Hwang I, Kim SY (2005) *Arabidopsis* calcium-dependent protein kinase AtCPK32 interacts with ABF4, a transcriptional regulator of abscisic acid-responsive gene expression, and modulates its activity. *Plant Physiology* **139**, 1750-1761

Chotikacharoensuk T, Arteca RN, Arteca JM (2006) Use of differential display for the identification of touch-induced genes from an ethylene-insensitive *Arabidopsis* mutant and partial characterization of these genes. *Journal of Plant Physiology* **163 (12)**, 1305-1320

Christodoulou J, Malmendal A, Harper JF, Chazin WJ (2004) Evidence for differing roles for each lobe of the calmodulin-like domain in a calcium-dependent protein kinase. *The Journal of Biological Chemistry* **279 (28)**, 29092-29100

Chung E, Park JM, Oh SK, Joung YH, Lee S, Choi D (2004) Molecular and biochemical characterization of the *Capsicum annuum* calcium-dependent protein kinase 3 (*CaCDPK3*) gene induced by abiotic and biotic stresses. *Planta* **220 (2)**, 286-95

Coca M, San Segundo B (2010) AtCPK1 calcium-dependent protein kinase mediates pathogen resistance in *Arabidopsis*. *Plant Journal* **63 (3)**, 526-540

Contreras-Porcia L, Dennett G, González A, Vergara E, Medina C, Correa JA, Moenne A (2011) Identification of copper-induced genes in the marine alga *Ulva compressa* (Chlorophyta). *Marine Biotechnology* **13 (3)**, 544-56

Dammann C, Ichida A, Hong B, Romanowsky SM, Hrabak EM, Harmon AC, Pickard BG, Harper JF (2003) Subcellular targeting of nine calcium-dependent protein kinase isoforms from *Arabidopsis*. *Plant Physiology* **132**, 1840-1848

Davletova S, Mesyaros T, Miskolczi P, Oberschall A, Torok K, Magyar Z, Dudits S, Deak M (2001) Auxin and heat shock activation of a novel member of the calmodulin-like domain protein kinase gene family in cultured alfalfa cells. *Journal of Experimental Botany* **52**, 215-221

Ehrhardt DW, Wais R, Long SR (1996) Calcium spiking in plant root hairs responding to *Rhizobium* nodulation signals. *Cell* **85**, 673-681

Ellard-Ivey M, Hopkins RB, White TJ, Lomax TL (1999) Cloning, expression and N-terminal myristoylation of *CpCPK1*, a calcium-dependent protein kinase from zucchini (*Cucurbita pepo* L.). *Plant Molecular Biology* **39**, 199-208

Estruch JJ, Kadwell S, Merlin E, Crossland L (1994) Cloning and characterization of a maize pollen-specific calcium-dependent calmodulin-independent protein kinase. *Proceedings of the National Academy of Sciences USA* **91**, 8837-8841

Evans NH, McAinsh MR, Hetherington AM (2001) Calcium oscillations in higher plants. *Current Opinion in Plant Biology* **4**, 415-420

Franz S, Ehlert B, Liese A, Kurth J, Cazalé AC, Romeis T (2011) Calcium-dependent protein kinase CPK21 functions in abiotic stress response in *Arabidopsis thaliana*. *Molecular Plant* **4 (1)**, 83-96

Frattini M, Morello L, Breviario D (1999) Rice calcium-dependent protein kinase isoforms OsCDPK2 and OsCDPK11 show different responses to light and different expression patterns during seed development. *Plant Molecular Biology* **41 (6)**, 753-764

Freymark G, Diehl T, Miklis M, Romeis T, Panstruga R (2007) Antagonistic control of powdery mildew host cell entry by barley calcium-dependent protein kinases (CDPKs). *Molecular Plant-Microbe Interactions* **20 (10)**, 1213-1221

Fukazawa J, Nakata M, Ito T, Matsushita A, Yamaguchi S, Takahashi Y (2011) bZIP transcription factor RSG controls the feedback regulation of NtGA20ox1 via intracellular localization and epigenetic mechanism. *Plant Signaling and Behavaviour* **6 (1)**, 26-28

Fukazawa J, Nakata M, Ito T, Yamaguchi S, Takahashi Y (2010) The transcription factor RSG regulates negative feedback of NtGA20ox1 encoding GA 20-oxidase. *Plant Journal* **62 (6)**, 1035-1045

Fukazawa J, Sakai T, Ishida S, Yamaguchi I, Kamiya Y, Takahashi Y (2000) Repression of shoot growth, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. *Plant Cell* **12 (6)**, 901-915

Gargantini P, Gonzalez-Rizzo S, Chinchilla D, Raices M, Giammaria V, Ulloa RM, Frugier F, Crespi MD (2006) A CDPK isoform participates in the regulation of nodule number in *Medicago truncatula*. *Plant Journal* **48**, 843-856

Gargantini PR, Giammaria V, Grandellis C, Feingold SE, Maldonado S, Ulloa RM (2009) Genomic and functional characterization of StCDPK1. *Plant Molecular Biology* **70 (1-2)**, 153-172

Gargantini PR, Gonzalez-Rizzo S, Chinchilla D, Raices M, Giammaria V, Ulloa RM, Frugier F, Crespi MD (2006) A CDPK isoform participates in the regulation of nodule number in *Medicago truncatula*. *Plant Journal* **48 (6)**, 843-856

Geiger D, Scherzer S, Mumm P, Marten I, Ache P, Matschi S, Liese A, Wellmann C, Al-Rasheid KA, Grill E, Romeis T, Hedrich R (2010) Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct Ca^{2+} affinities. *Proceedings of the National Academy of Sciences USA* **107 (17)**, 8023-8028

Giammaria V, Grandellis C, Bachmann S, Gargantini PR, Feingold SE, Bryan G, Ulloa RM (2011) *StCDPK2* expression and activity reveal a highly responsive potato calcium-dependent protein kinase involved in light signalling. *Planta* **233 (3)**, 593-609

Glinski M, Romeis T, Witte CP, Wienkoop S, Weckwerth W (2003) Stable isotope labeling of phosphopeptides for multiparallel kinase target analysis and identification of phosphorylation sites. *Rapid Communications in Mass Spectrometry* **17 (14)**, 1579-1584

Grant M, Brown I, Adams S, Knight M, Ainslie A, Mansfield J (2000) The RPM1 plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. *Plant Journal* **23**, 441-450

Guo P, Baum M, Grando S, Ceccarelli S, Bai G, Li R, von Korff M, Varshney RK, Graner A, Valkoun J (2009) Differentially expressed genes between drought-tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. *Journal of Experimental Botany* **60 (12)**, 3531-3544

Gupta V, Khurana R, Tyagi AK (2007) Promoters of two anther-specific genes confer organ-specific gene expression in a stage-specific manner in transgenic systems. *Plant Cell Reports* **26 (11)**, 1919-1931

Harmon AC, Gribskov M, Gubrium E, Harper JF (2001) The CDPK superfamily of protein kinases. *New Phytologist* **151**, 175-183

Harmon AC, Putnam-Evans C, Cormier MJ (1987) A calcium-dependent but calmodulin-independent protein kinase from soybean. *Plant Physiology* **83**, 830-837

Harmon AC, Yoo BC, McCaffery C (1994) Pseudosubstrate inhibition of CDPK, a protein kinase with a calmodulin-like domain. *Biochemistry* **33 (23)**, 7278-7287

Harper JF, Breton G, Harmon A (2004) Decoding Ca^{2+} signals through plant protein kinases. *Annual Review of Plant Biology* **55**, 263-288

Harper JF, Huang JF, Lloyd SJ (1994) Genetic identification of an autoinhibitor in CDPK, a protein kinase with a calmodulin-like domain. *Biochemistry* **33 (23)**, 7267-7277

Harper JF, Sussman MR, Schaller GE, Putnam-Evans C, Charbonneau H, Harmon AC (1991) A calcium-dependent protein kinase with a regulatory domain similar to calmodulin. *Science* **252 (1)**, 951-954

Hassan A, Okuta T, Kato M, Hatsugai N, Sano Y, Ishimori T, Okazaki K, Doullah MA, Shah MM (2012) Alternaric acid stimulates phosphorylation of His-tagged RiCDPK2, a calcium-dependent protein kinase in potato plants. *Genetics and Molecular Research* **11 (3)**, 2381-2389

Hegeman AD, Rodriguez M, Han BW, Uno Y, Phillips GN, Hrabak EM, Uno Y, Phillips GN, Hrabak EM, Cushman JC, Harper JF, Harmon AC, Sussman MR (2006) A phyloproteomic characterization of *in vitro* autophosphorylation in calcium-dependent protein kinases. *Proteomics* **6**, 3649-3664

Helper PK (2005) Calcium: A central regulator of plant growth and development. *Plant Cell* **17**, 2142-2155

Hetherington AM, Trewavas A (1982) Calcium-dependent protein kinase in pea shoot membranes. *FEBS Letters* **145**, 67-71

Holdaway-Clarke TL, Feijo JA, Hackett GR, Kunkel JG, Hepler PK (1997) Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx is delayed. *Plant Cell* **9**, 1999-2010

Honys D, Twell D (2004) Transcriptome analysis of haploid male gametophyte development in *Arabidopsis*. *Genome Biology* **5**, R85

Hrabak EM, Chan CW, 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 Physiology* **132**, 666-680

Huang JF, Teyton L, Harper JF (1996) Activation of a Ca^{2+}-dependent protein kinase involves intramolecular binding of a calmodulin-like regulatory domain. *Biochemistry* **35 (40)**, 13222-13230

Huang QS, Wang HY, Gao P, Wang GY, Xia GX (2008) Cloning and characterization of a calcium dependent protein kinase gene associated with cotton fiber development. *Plant Cell Reporter* **27 (12)**, 1869-1875

Hubbard KE, Siegel RS, Valerio G, Brandt B, Schroeder JI (2011) Abscisic acid and CO_2 signalling via calcium sensitivity priming in guard cells, new CDPK mutant phenotypes and a method for improved resolution of stomatal stimulus-response analyses. *Annals of Botany* **109 (1)**, 5-17

Igarashi D, Ishida S, Fukazawa J, Takahashi Y (2001) 14-3-3 proteins regulate intracellular localization of the bZIP transcriptional activator RSG. *Plant Cell* **13 (11)**, 2483-2497

Ishida S, Fukazawa J, Yuasa T, Takahashi Y (2004) Involvement of 14-3-3 signaling protein binding in the functional regulation of the transcriptional activator REPRESSION OF SHOOT GROWTH by gibberellins. *Plant Cell* **16 (10)**, 2641-2651

Ishida S, Yuasa T, Nakata M, Takahashi Y (2008) A tobacco calcium-dependent protein kinase, CDPK1, regulates the transcription factor REPRESSION OF SHOOT GROWTH in response to gibberellins. *Plant Cell* **20 (12)**, 3273-3288

Ito T, Nakata M, Fukazawa J, Ishida S, Takahashi Y (2010) Alteration of substrate specificity: the variable N-terminal domain of tobacco Ca^{2+}-dependent protein kinase is important for substrate recognition. *Plant Cell* **22 (5)**, 1592-1604

Ivashuta S, Liu J, Liu J, Lohar DP, Haridas S, Bucciarelli B, VandenBosch KA, Vance CP, Harrison MJ, Gantt JS (2005) RNA interference identifies a calcium-dependent protein kinase involved in *Medicago truncatula* root development. *Plant Cell* **17 (11)**, 2911-2921

Iwanaga T, Tsutsumi R, Noritake J, Fukata Y, Fukata M (2009) Dynamic protein palmitoylation in cellular signaling. *Progress in Lipid Research* **48**, 117-127

Jain M, Pathak BP, Harmon AC, Tillman BL, Gallo M (2011) Calcium dependent protein kinase (CDPK) expression during fruit development in cultivated peanut (*Arachis hypogaea*) under Ca^{2+}-sufficient and -deficient growth regimens. *Journal of Plant Physiology* **168 (18)**, 2272-2277

Katou S, Yamamoto A, Yoshioka H, Kawakita K, Doke N (2003) Functional analysis of potato mitogen-activated protein kinase kinase, StMEK1. *Journal of General Plant Pathology* **69**, 161-168

Katou S, Yoshioka H, Kawakita K, Rowland O, Jones JDG, Mori H, Doke N (2005) Involvement of PPS3 phosphorylated by elicitor-responsive mitogen-activated protein kinases in the regulation of plant cell death. *Plant Physiology* **139**, 1914-1926

Kawasaki T, Hayashida N, Baba T, Shinozaki K, Shimada H (1993) The gene encoding a calcium-dependent protein kinase located near the sbe1 gene encoding starch branching enzyme I is specifically expressed in developing rice seeds. *Gene* **129 (2)**, 183-189

Kawasaki T, Okumura S, Kishimoto N, Shimada H, Higo K, Ichikawa N (1999) RNA maturation of the rice SPK gene may involve *trans*-splicing. *Plant Journal* **18 (6)**, 625-632

Kim TH, Böhmer M, Hu H, Nishimura N, Schroeder JI (2011) Guard cell signal transduction network: Advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. *Annual Review of Plant Biology* **61**, 561-591

Kiselev KV, Grishchenko OV, Zhuravlev YN (2009) CDPK gene expression in salt tolerant *rolB* and *rolC* transformed cell cultures of *Panax ginseng*. *Biologia Plantarum* **54 (4)**, 621-630

Knight H (2000) Calcium signaling during abiotic stress in plants. *International Review of Cytology* **195**, 269-324

Knight H, Knight MR (2001) Abiotic stress signalling pathways: Specificity and cross-talk. *Trends in Plant Science* **6 (6)**, 262-267

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

Komatsu S, Yang G, Khan M, Onodera H, Toki S, Yamaguchi M (2007) Over-expression of calcium-dependent protein kinase 13 and calreticulin interacting protein 1 confers cold tolerance on rice plants. *Molecular Genetics and Genomics* **277**, 713-723

Kumar KG, Ullanat R, Jayabaskaran C (2004) Molecular cloning, characterization, tissue-specific and phytohormone-induced expression of calcium-dependent protein kinase gene in cucumber (*Cucumis sativus* L.). *Journal of Plant Physiology* **161**, 1061-1071

Kwak JM, Moon JH, Murata Y, Kuchitsu K, Leonhardt N, DeLong A, Schroeder JI (2002) Disruption of a guard cell-expressed protein phosphatase 2A regulatory subunit, *RCN1*, confers abscisic acid insensitivity in *Arabidopsis*. *Plant Cell* **14**, 2849-2861

Lanteri ML, Pagnussat GC, Lamattina L (2006) Calcium and calcium-dependent protein kinases are involved in nitric oxide- and auxin-induced adventitious root formation in cucumber. *Journal of Experimental Botany* **57 (6)**, 1341-1351

Lee SS, Cho HS, Yoon GM, Ahn JW, Kim HH, Pai HS (2003) Interaction of NtCDPK1 calcium-dependent protein kinase with NtRpn3 regulatory subunit of the 26S proteasome in *Nicotiana tabacum*. *Plant Journal* **33 (5)**, 825-840

Li A, Wang X, Leseberg CH, Jia J, Mao L (2008) Biotic and abiotic stress responses through calcium-dependent protein kinase (CDPK) signaling in wheat (*Triticum aestivum* L.). *Plant Signaling and Behaviour* **3 (9)**, 654-656

Li J, Lee YRJ, Assmann SM (1998) Guard cells possess a calcium-dependent protein kinase that phosphorylates the KAT1 potassium channel. *Plant Physiology* **116**, 785-795

Lima L, Seabra A, Melo P, Cullimore J, Carvalho H (2006a) Post-translational regulation of cytosolic glutamine synthetase of *Medicago truncatula*. *Journal of Experimental Botany* **57 (11)**, 2751-2761

Lima L, Seabra A, Melo P, Cullimore J, Carvalho H (2006b) Phosphorylation and subsequent interaction with 14-3-3 proteins regulate plastid glutamine synthetase in *Medicago truncatula*. *Planta* **223 (3)**, 558-567

Lino B, Carrillo-Rayas MT, Chagolla A, González de la Vara LE (2006) Purification and characterization of a calcium-dependent protein kinase from beetroot plasma membranes. *Planta* **225 (1)**, 255-268

Liu G, Chen J, Wang X (2006) *VfCPK1*, a gene encoding calcium-dependent protein kinase from *Vicia faba*, is induced by drought and abscisic acid. *Plant Cell Environment* **29 (11)**, 2091-2099

Llop-Tous I, Dominquez-Puigjaner E, Vendrel lM (2002) Characterization of a strawberry cDNA clone homologous to calcium-dependent protein kinases that is expressed during ripening and affected by low temperature. *Journal of Experimental Botany* **53**, 2283-2285

Lu B, Ding R, Zhang L, Yu X, Huang B, Chen W (2006) Molecular cloning and characterization of a novel calcium-dependent protein kinase gene liCPK2 responsive to polyploidy from tetraploid *Isatis indigotica*. *Journal of Biochemistry and Molecular Biology* **39 (5)**, 607-617

Lu SX, Hrabak EM (2002) An *Arabidopsis* calcium-dependent protein kinase is associated with the endoplasmic reticulum. *Plant Physiology* **128 (3)**, 1008-1021

Ludwig AA, Romeis T, Jones JDG (2004) CDPK-mediated signalling pathways: Specificity and cross-talk. *Journal of Experimental Botany* **55 (395)**, 181-188

Ludwig AA, Saitoh H, Felix G, Freymark G, Miersch O, Wasternack C, Boller T, Jones JD, Romeis T (2005) Ethylene-mediated cross-talk between calcium-dependent protein kinase and MAPK signaling controls stress responses in plants. *Proceedings of the National Academy of Sciences USA* **102**, 10736-10741

Ma SY, Wu WH (2007) AtCPK23 functions in *Arabidopsis* responses to drought and salt stresses. *Plant Molecular Biology* **65 (4)**, 511-518

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics* **444**, 139-158

Mall TK, Dweikat I, Sato SJ, Neresian N, Xu K, Ge Z, Wang D, Elthon T, Clemente T (2011) Expression of the rice *CDPK-7* in sorghum: Molecular and phenotypic analyses. Plant Molecular Biology **75 (4-5)**, 467-479

Martín ML, Busconi L (2000) Membrane localization of a rice calcium-dependent protein kinase (CDPK) is mediated by myristoylation and palmitoylation. *Plant Journal* **24 (4)**, 429-435

Martín ML, Busconi L (2001) A rice membrane-bound calcium-dependent protein kinase is activated in response to low temperature. *Plant Physiology* **125 (3)**, 1442-1449

Martínez-Noël G, Nagaraj VJ, Caló G, Wiemken A, Pontis HG (2007) Sucrose regulated expression of a Ca^{2+}-dependent protein kinase (*TaCDPK1*) gene in excised leaves of wheat. *Plant Physiology and Biochemistry* **45 (6-7)**, 410-419

McCubbin AG, Ritchie SM, Swanson SJ, Gilroy S (2004) The calcium-dependent protein kinase *HvCDPK1* mediates the gibberellic acid response of the barley aleurone through regulation of vacuolar function. *Plant Journal* **39 (2)**, 206-218

Mehlmer N, Wurzinger B, Stael S, Hofmann-Rodrigues D, Csaszar E, Pfister B, Bayer R, Teige M (2010) The Ca^{2+}-dependent protein kinase CPK3 is required for MAPK-independent salt-stress acclimation in *Arabidopsis*. *Plant Journal* **63**, 484-498

Messerli MA, Robinson KR (1997) Tip localized Ca^{2+} pulses are coincident with peak pulsatile pollen growth rates in pollen tubes of *Lilium longiflorum*. *Journal of Cell Science* **112**, 1497-1509

Milla MAR, Townsend J, Chang IF, Cushman JC (2006b) The *Arabidopsis* AtDi19 gene family encodes a novel type of Cys2/His2 zinc-finger protein implicated in ABA-independent dehydration, high-salinity stress and light signaling pathways. *Plant Molecular Biology* **61**, 13-30

Milla MAR, Uno Y, Chang IF, Townsend J, Maher EA, Quilici D, Cushman JC (2006a) A novel yeast two-hybrid approach to identify CDPK substrates:

Characterization of the interaction between CPK11 and AtDi19, a nuclear zinc finger protein. *FEBS Letters* **580**, 904-911

Monroy AF, Dhindsa RS (1995) Low-temperature signal transduction: Induction of cold acclimation-specific genes of alfalfa by calcium at 25°C. *Plant Cell* **7 (3)**, 321-331

Morello L, Bardini M, Cricrì M, Sala F, Breviario D (2006) Functional analysis of DNA sequences controlling the expression of the rice *OsCDPK2* gene. *Planta* **223 (3)**, 479-491

Morello L, Frattini M, Giani S, Christou P, Breviario D (2000) Overexpression of the calcium-dependent protein kinase *OsCDPK2* in transgenic rice is repressed by light in leaves and disrupts seed development. *Transgenic Research* **9**, 453-462

Morello L, Giani S, Coraggio I, Breviario D (1993) Rice membranes contain a calcium-dependent protein kinase activity with biochemical features of animal protein kinase C. *Biochemistry and Biophysics Research Communication* **197**, 55-61

Mori IC, Murata Y, Yang Y, Munemasa S, Wang YF, Andreoli S, Tiriac H, Alonso JM, Harper JF, Ecker JR, Kwak JM, Schroeder JI (2006) CDPKs CPK6 and CPK3 function in ABA regulation of guard cell S-type anion- and Ca^{2+}-permeable channels and stomatal closure. *PLoS Biology* **4 (10)**, e327

Munemasa S, Mori IC, Murata Y (2011) Methyl jasmonate signaling and signal crosstalk between methyl jasmonate and abscisic acid in guard cells. *Plant Signaling and Behaviour* **6 (7)**, 939-941

Munemasa S, Oda K, Watanabe-Sugimoto M, Nakamura Y, Shimoishi Y, Murata Y (2007) The coronatine-insensitive 1 mutation reveals the hormonal signaling interaction between abscisic acid and methyl jasmonate in *Arabidopsis* guard cells: Specific impairment of ion channel activation and second messenger production. *Plant Physiology* **143**, 1398-1407

Muñiz García MN, Giammaria V, Grandellis C, Téllez-Iñón MT, Ulloa RM, Capiati DA (2011) Characterization of StABF1, a stress-responsive bZIP transcription factor from *Solanum tuberosum* L. that is phosphorylated by StCDPK2 *in vitro*. *Planta* **235 (4)**, 761-778

Murillo I, Jaeck E, Cordero MJ, San Segundo B (2001) Transcriptional activation of a maize calcium-dependent protein kinase gene in response to fungal elicitors and infection. *Plant Molecular Biology* **45**, 145-158

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 Journal* **59 (4)**, 528-539

Pagnussat GC, Fiol DF, Salerno GL (2002) A CDPK type protein kinase is involved in rice SPS light modulation. *Physiologia Plantarum* **115**, 183-189

Patharkar OR, Cushman JC (2000) A stress-induced calcium-dependent protein kinase from *Mesembryanthemum crystallinum* phosphorylates a two-component pseudo-response regulator. *Plant Journal* **24 (5)**, 679-691

Patharkar OR, Cushman JC (2006) A novel coiled-coil protein co-localizes and interacts with a calcium-dependent protein kinase in the common ice plant during low-humidity stress. *Planta* **225 (1)**, 57-73

Pical C, Fredlund KM, Petit PX, Sommarin M, Moller IM (1993) The outer membrane of plant mitochondria contains a calcium-dependent protein kinase and multiple phosphoproteins. *FEBS Letters* **336**, 347-351

Putnam-Evans C, Harmon AC, Cormier MJ (1986) Calcium-dependent protein phosphorylation in suspension-cultured soybean cells. In: Trewavas AJ (Ed) *Molecular and Cellular Aspects Calcium in Plant Development*, Plenum, New York, pp 99-106

Putnam-Evans C, Harmon AC, Palevitz BA, Fechheimer M, Cormier MJ (1989) Calcium-dependent protein kinase is localized with F-actin in plant cells. *Cell Motility and the Cytoskeleton* **12**, 12-22

Quintero FJ, Martinez-Atienza J, Villalta I, Jiang X, Kim WY, Ali Z, Fujii H, Mendoza I, Yun DJ, Zhu JK, Pardo JM (2011) Activation of the plasma membrane Na/H antiporter Salt-Overly-Sensitive 1 (SOS1) by phosphorylation of an auto-inhibitory C-terminal domain. *Proceedings of the National Academy of Sciences USA* **108 (6)**, 2611-2616

Raices M, Chico M, Tellez T, Ulloa R (2001) Molecular characterization of StCDPK1, a calcium-dependent protein kinase from *Solanum tuberosum* that is induced at the onset of tuber development. *Plant Molecular Biology* **46**, 591-601

Raices M, Gargantini PR, Chinchilla D, Martin C, Trllez-Inon M, Ulloa RM (2003) Regulation of CDPK isoforms during tuber development. *Plant Molecular Biology* **52**, 1011-1024

Raichaudhuri A, Bhattacharyya R, Chaudhury S, Chakrabarti P, Das Gupta M (2006) Domain analysis of a groundnut cdpk: Nuclear localisation sequence in the junction domain is coupled with nonconsensus calcium binding domains. *Journal of Biological Chemistry* **281 (15)**, 10399-10409

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*). *Molecular Genetics and Genomics* **278**, 493-505

Reddy ASN (2001) Calcium: Silver bullet in signaling. *Plant Science* **160**, 381-404

Ritchie S, Gilroy S (1998) Calcium-dependent protein phosphorylation may mediate the gibberellic acid response in barley aleurone. *Plant Physiology* **116**, 765-776

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

Rudd JJ, Franklin-Tong VE (2001) Unravelling response-specificity in Ca^{2+} signalling pathways in plant cells. *New Phytologist* **151**, 7-33

Rutschmann F, Stalder U, Piotrowski M, Oecking C, Schaller A (2002) *LeCPK1*, a calcium-dependent protein kinase from tomato: Plasma membrane targeting and biochemical characterization. *Plant Physiol*ogy **129**, 156-168

Sachs JN, Engelman DM (2006) Introduction to the membrane protein reviews: The interplay of structure, dynamics, and environment in membrane protein function. *Annual Review of Biochemistry* **75**, 707-712

Saijo Y, Hata S, Kyozuka J, Shimamoto K, Izui K (2000) Over-expression of a single Ca^{2+}-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant Journal* **23**, 319-327

Saijo Y, Hata S, Sheen J, Izui K (1997) cDNA cloning and prokaryotic expression of maize calcium-dependent protein kinases. *Biochimica et Biophysica Acta* **1350**, 109-114

Saijo Y, Kinoshita N, Ishiyama K, Hata S, Kyozuka J, Hayakawa T, Nakamura T, Shimamoto K, Yamaya T, Izui K (2001) A Ca^{2+}-dependent protein kinase that endows rice plants with cold- and salt-stress tolerance functions in vascular bundles. *Plant Cell Physiology* **42**, 1228-1233

Saito N, Munemasa S, Nakamura Y, Shimoishi Y, Mori IC, Murata Y (2008) Roles of RCN1, regulatory A subunit of protein phosphatase 2A, in methyl jasmonate signaling and signal crosstalk between methyl jasmonate and abscisic acid. *Plant Cell Physiology* **49 (9)**, 1396-1401

Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. *Plant Cell* **11**, 691-706

Sanders D, Pelloux J, Brownlee C, Harper JF (2002) Calcium at the crossroads of signalling. *Plant Cell* **14**, S401-S417

Sheen J (1996) Ca^{2+}-dependent protein kinases and stress signal transduction in plants. *Science* **274**, 1900-1902

Shimada H, Koishihara H, Saito Y, Arashima Y, Furukawa T, Hayashi H (2004) A rice antisense SPK transformant that lacks the accumulation of seed storage substances shows no correlation between sucrose concentration in phloem sap and demand for carbon sources in the sink organs. *Plant Cell Physiology* **45 (8)**, 1105-1109

Shkryl YN, Veremeichik GN, Bulgakov VP, Zhuravlev YN (2011) Induction of anthraquinone biosynthesis in *Rubia cordifolia* cells by heterologous expression of a calcium-dependent protein kinase gene. *Biotechnology and Bioengineering* **108 (7)**, 1734-1738

Song WY, Zhang ZB, Shao HB, Guo XL, Cao HX, Zhao HB, Fu ZY, Hu XJ (2008) Relationship between calcium decoding elements and plant abiotic-stress resistance. *International Journal of Biological Sciences* **4 (2)**, 116-125

Stael S, Bayer RG, Mehlmer N, Teige M (2011) Protein N-acylation overrides differing targeting signals. *FEBS Letters* **585 (3)**, 517-522

Syam PSR, Chelliah J (2006a) Expression and localization of calcium-dependent protein kinase isoforms in chickpea. *Journal of Plant Physiology* **163 (11)**, 1135-1149

Syam PSR, Chelliah J (2006b) Heterologous expression and biochemical characterization of two calcium-dependent protein kinase isoforms CaCPK1 and CaCPK2 from chickpea. *Journal of Plant Physiology* **63 (11)**, 1083-1093

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

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

Szczegielniak J, Liwosz A, Jurkowski I, Loog M, Dobrowolska G, Ek P, Harmon AC, Muszyńska G (2000) Calcium-dependent protein kinase from maize seedlings activated by phospholipids. *European Journal of Biochemistry* **267 (12)**, 3818-3827

Taniguchi H (1999) Protein myristoylation in protein-lipid and protein-protein interactions. *Biophysical Chemistry* **82**, 129-137

Taylor LP, Hepler PK (1997) Pollen germination and tube growth. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 461-491

Tsai TM, Chen YR, Kao TW, Tsay WS, Wu CP, Huang DD, Chen WH, Chang CC, Huang HJ (2007) *PaCDPK1*, a gene encoding calcium-dependent protein kinase from orchid, *Phalaenopsis amabilis*, is induced by cold, wounding, and pathogen challenge. *Plant Cell Reports* **26 (10)**, 1899-1908

Ullanat R, Jayabaskaran C (2002) Distinct light-, cytokinin- and tissue-specific regulation of calcium-dependent protein kinase gene expression in cucumber (*Cucumis sativus*). *Plant Science* **162**, 153-163

Ulloa RM, Raices M, Macintosh GC, Maldonado S, Tellez-Inon M (2002) Jasmonic acid affects plant morphology and calcium-dependent protein kinase expression and activity in *Solanum tuberosum*. *Physiologia Plantarum* **115**, 417-427

Uno Y, Miguel A, Milla R, Maher E, Cushman JC (2009) Identification of proteins that interact with catalytically active calcium-dependent protein kinases from *Arabidopsis*. *Molecular Genetics and Genomics* **281**, 375-390

Urao T, Katagiri T, Mizoguchi T, Yamaguchi-Shinozaki K, Hayashida N,

Shinozaki K (1994) Two genes that encode Ca^{2+}-dependent protein kinases are induced by drought and high-salt stresses in *Arabidopsis thaliana*. *Molecular and General Genetics* **244**, 331-340

Wan B, Lin Y, Mou T (2007) Expression of rice Ca^{2+}-dependent protein kinases (CDPKs) genes under different environmental stresses. *FEBS Letters* **581**, 1179-1189

Wang CW, Chen WC, Lin LJ, Lee CT, Tseng TH, Leu WM (2011) OIP30, a RuvB-Like DNA Helicase 2, is a potential substrate for the pollen-predominant OsCPK25/26 in rice. *Plant Cell Physiology* **52 (9)**, 1641-1656

Wang H, Mei W, Qin Y, Zhu Y (2011) 1-Aminocyclopropane-1-carboxylic acid synthase 2 is phosphorylated by calcium-dependent protein kinase 1 during cotton fiber elongation. *Acta Biochimica et Biophysica Sinica* **43 (8)**, 654-661

Wang Y, Zhang M, Ke K, Lu YT (2005) Cellular localization and biochemical characterization of a novel calcium-dependent protein kinase from tobacco. *Cell Research* **15 (8)**, 604-612

Wei LQ, Xu WY, Deng ZY, Su Z, Xue Y, Wang T (2010) Genome-scale analysis and comparison of gene expression profiles in developing and germinated pollen in *Oryza sativa. BMC Genomics* **11**, 338

Williamson RE, Ashley CC (1982) Free Ca^{2+} and cytoplasmic streaming in the alga *Chara*. *Nature* **296**, 647-651

Witte CP, Keinath N, Dubiella U, Demoulière R, Seal A, Romeis T (2010) Tobacco calcium-dependent protein kinases are differentially phosphorylated *in vivo* as part of a kinase cascade that regulates stress response. *The Journal of Biological Chemistry* **285 (13)**, 9740-9748

Wyn Jones RG, Lunt OR (1967) The function of calcium in plants. *Botanical Review* **33**, 407-426

Xing T, Wang XJ, Malik K, Miki BL (2001) Ectopic expression of an *Arabidopsis* calmodulin-Like domain protein kinase-enhanced NADPH oxidase activity and oxidative burst in tomato protoplasts. *Molecular Plant-Microbe Interactions* **14 (10)**, 1261-1264

Xu J, Tian YS, Peng RH, Xiong AS, Zhu B, Jin XF, Gao F, Fu XY, Hou XL, Yao QH (2010) AtCPK6, a functionally redundant and positive regulator involved in salt/drought stress tolerance in *Arabidopsis*. *Planta* **231 (6)**, 1251-1260

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annual Review of Plant Biology* **57**, 781-803

Yang G, Shen S, Yang S, Komatsu S (2003) OsCDPK13, a calcium-dependent protein kinase gene from rice, is induced in response to cold and gibberellin. *Plant Physiology and Biochemistry* **41**, 369-374

Ye S, Wang L, Xie W, Wan B, Li X, Lin Y (2009) Expression profile of calcium-dependent protein kinase (CDPKs) genes during the whole lifespan and under phytohormone treatment conditions in rice (*Oryza sativa* L. ssp. indica). *Plant Molecular Biology* **70 (3)**, 311-325

Yoo B, Harmon AC (1996) Intramolecular binding contributes to the activation of CDPK, a protein kinase with a calmodulin-like domain. *Biochemistry* **2960 (96)**, 12029-12037

Yoo BC, Lee JY, Lucas WJ (2002) Analysis of the complexity of protein kinases within the phloem sieve tube system. Characterization of *Cucurbita maxima* calmodulin-like domain protein kinase 1. *Journal of Biological Chemistry* **277 (18)**, 15325-15332

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 Molecular Biology* **39 (5)**, 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

Yoshioka H, Mase K, Yoshioka M, Kobayashi M, Asai S (2011) Regulatory mechanisms of nitric oxide and reactive oxygen species generation and their role in plant immunity. *Nitric oxide* **25 (2)**, 216-221

Yu XC, Li MJ, Gao GF, Feng HZ, Geng XQ, Peng CC, Zhu SY, Wang XL, Shen YY, Zhang DP (2006) Abscisic acid stimulates a calcium-dependent protein kinase in grape berry. *Plant Physiology* **140**, 558-579

Yu XC, Zhu SY, Gao GF, Wang XJ, Zhao R, Zou KQ, Wang XF, Zhang XY, Wu FQ, Peng CC, Zhang DP (2007) Expression of a grape calcium-dependent protein kinase ACPK1 in *Arabidopsis thaliana* promotes plant growth and confers abscisic acid-hypersensitivity in germination, postgermination growth, and stomatal movement. *Plant Molecular Biology* **64 (5)**, 531-538

Zhang T, Wang Q, Chen X, Tian C, Wang X, Xing T, Li Y, Wang Y (2005) Cloning and biochemical properties of CDPK gene *OsCDPK14* from rice. *Journal of Plant Physiology* **162**, 1149-1159

Zhang XS, Choi JH (2001) Molecular evolution of calmodulin-like domain protein kinases (CDPKs) in plants and protists. *Journal of Molecular Evolution* **53 (3)**, 214-224

Zhao R, Sun HL, Mei C, Wang XJ, Yan L, Liu R, Zhang XF, Wang XF, Zhang DP (2011) The *Arabidopsis* Ca^{2+}-dependent protein kinase CPK12 negatively regulates abscisic acid signaling in seed germination and post-germination growth. *New Phytologist* **192 (1)**, 61-73

Zheng H, von Mollard GF, Kovaleva V, Stevens TH, Raikhel NV (1999) The plant vesicle-associated SNARE AtVTI1a likely mediates vesicle transport from the *trans*-Golgi network to the prevacuolar compartment. *Molecular Biology of the Cell* **10 (7)**, 2251-2264

Zhu JH, Chen X, Chang WJ, Tian WM, Zhang ZL (2010) Molecular characterization of *HbCDPK1*, an ethephon-induced calcium-dependent protein kinase gene of *Hevea brasiliensis*. *Bioscience, Biotechnology, and Biochemistry* **74 (11)**, 2183-2188

Zhu SY, Yu XC, Wang XJ, Zhao R, Li Y, Fan RC, Shang Y, Du SY, Wang XF, Wu FQ, Xu YH, Zhang XY, Zhang DP (2007) Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in *Arabidopsis*. *Plant Cell* **19**, 3019-3036

Zou JJ, Wei FJ, Wang C, Wu JJ, Ratnasekera D, Liu WX, Wu WH (2010) *Arabidopsis* calcium-dependent protein kinase CPK10 functions in abscisic acid-and Ca^{2+}-mediated stomatal regulation in response to drought stress. *Plant Physiology* **154 (3)**, 1232-1243