

Role of Ca²⁺-mediated signaling in potato tuberization: An overview

Akula NOOKARAJU^{1,3}, Shashank K. PANDEY^{1,3}, Chandrama P. UPADHYAYA^{1,4}, Jeon Jae HEUNG², Hyun S. KIM², Se Chul CHUN¹, Doo Hwan KIM¹, and Se Won PARK^{1,*}

¹Department of Molecular Biotechnology, Konkuk University, Seoul - 143 701, Korea

²Korean Research Institute of Bioscience and Biotechnology, Daejeon, Korea

³Department of Bioenergy Science and Technology, Chonnam National University, Gwangju, Korea

⁴Department of Botany, School of Life Sciences, GG Central University, Bilaspur, CG, India

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ABSTRACT. Potato tuberization represents the morphogenetic transition of underground shoot to tuber involving several biochemical and molecular changes under complex environmental, nutritional and endogenous regulation. Among the nutritional factors, the role of calcium in potato tuberization is documented in several earlier studies. Calcium is a major essential nutrient required for normal growth and development of plants. As a second messenger it plays a role in a number of fundamental cellular processes like cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell differentiation, photomorphogenesis, plant defense and various stress responses. Calcium in the cytosol regulates the activity of Ca²⁺-sensor proteins and these proteins will subsequently activate and/or modify the activity of target proteins in biological pathways. Also, cytosolic calcium regulates oxidative burst via calcium dependent protein kinases (CDPKs) and induces many intracellular signaling pathways. Studies suggest that Ca²⁺ and Ca²⁺-sensor protein calmodulin (CaM) have a role as signal molecules for tuber induction in potato. Also, a potato Ca²⁺-dependent protein kinase, StCDPK1, is reported to be transiently expressed in tuberizing stolons suggesting its possible involvement in potato tuberization by transcriptional activation of some of the tuberizing genes. Though Ca²⁺ and Ca²⁺-regulated proteins influence many developmental processes in plants, the exact molecular and biochemical mechanism of Ca²⁺-mediated signal pathways controlling potato tuberization is still not clear. This review sheds some light on the possible molecular mechanisms involved in the Ca²⁺-mediated signaling in potato tuberization.

Keywords: Calcium; CaM; CDPK; Tuberization; GA metabolism; Oxidative metabolism.

Abbreviations: ABA, abscisic acid; AQP, aquaporins; BA, 6-benzyl adenine; CaM, calmodulin; Ca²⁺/CaM, Ca²⁺-bound CaM; [Ca²⁺]_{cyt}, cytosolic Ca²⁺; CBL, calcineurin B-like proteins; CCaMK, chimeric Ca²⁺/CaM-dependent protein kinase; CDPK, Ca²⁺-dependent protein kinase; CIPK, CBL interacting protein kinases; CK, cytokinin; GA, gibberellic acid; LOX, lipoxygenase; JA, jasmonic acid; PCBP, potato CaM binding protein; SOD, superoxide dismutase; StCBP, *Solanum tuberosum* Ca²⁺/CaM-binding protein; TA, tuberonic acid; TAG, tuberonic acid glucoside; TFs, transcription factors; ZBF3, Z-box binding factor.

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*Corresponding author: E-mail: sewpark@konkuk.ac.kr; Phone: +82-2-450-3739.

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INTRODUCTION

Tuberization is a complex phenomenon involving a morphological transition of an underground shoot to stolon and subsequent tuber formation under complex environmental, nutritional and endogenous regulation (Figure 1). It comprises induction, initiation and growth of the stolon followed by the initiation and growth of the storage organ, tuber. Tuberization in potato serves dual function, as a storage organ and a mean to vegetative propagation. A dynamic change in the expression pattern of metabolic enzymes, endogenous growth regulators, protease inhibitors and accumulation of storage proteins, such as patatins, was observed at the onset of tuberization (Prat et al., 1990; Taylor et al., 1992a, b, 1993; Jackson et al., 1997). A wide variety of soil, environmental and hormonal stimuli are known to be involved in the induction of potato tuberization (Menzel, 1983, 1985; Vreugdenhil and Struik, 1989; Pelacho et al., 1994). Among the soil factors, calcium (Ca^{2+}) nutrition plays an important role in potato tuberization. Calcium is an essential component of the plant cell wall giving mechanical strength and providing the medium for normal transport and retention of other elements. It is an essential nutrient and has been shown to affect protein phosphorylation in plants (Budde and Chollet, 1988) through affecting the calcium-binding modulator proteins and protein kinases. Subsequently, protein kinases modulate the activity of many key enzymes through protein phosphorylation, a major mechanism involved in transduction of various Ca^{2+} signals (Sopory and Munshi, 1998).

Experiments with single-node leaf cuttings from induced potato (*Solanum tuberosum* L.) plants suggested the possible role of Ca^{2+} as a mediator of tuberization stimulus (Balamani et al., 1986). A few other studies demonstrated the influence of supplemental Ca^{2+} on tuberization of potato under field conditions (Ozgen et al., 2000, 2003, 2006; Ozgen and Palta, 2004; Chang et al., 2007) though the exact mechanism of its influence was not studied. Reports also suggested the possible involvement of Ca^{2+} -sensor proteins such as calmodulin (CaM) (Jena et al., 1989) and potato calcium dependent protein kinase (StCDPK1) in tuberization of potato (MacIntosh et al., 1996; Raices et al., 2001, 2003; Ganrgantini et al., 2009). In spite of evidences for the positive role of Ca^{2+} and various Ca^{2+} -regulated proteins in potato tuberization, the exact molecular mechanism of Ca^{2+} and Ca^{2+} -induced signal pathways controlling tuberization is not studied well. In this review we endeavor to provide an update on the recent advances and discussion on various aspects of Ca^{2+} -mediated signaling in potato tuberization.

NUTRITIONAL AND PHYSIOLOGICAL ROLES OF Ca^{2+} IN PLANTS

Calcium is a major nutrient required for normal growth and development of plants. As a divalent cation (Ca^{2+}), it has structural roles in the cell wall and cell membranes as a counter cation for anions in the vacuoles (White and Broadley, 2003), and acts as intracellular messenger in the cytosol (Marschner, 1995). The most striking use of Ca^{2+} ions as a structural element in plants occurs in the marine coccolithophores, which use Ca^{2+} to form the calcium carbonate plates with which they are covered. In plants, Ca^{2+} is usually stored as Ca-oxalate crystals in plastids. Calcium is critical for plant cells providing strong structural rigidity by forming cross-links within the pectin polysaccharide matrix (Easterwood, 2002). Calcium deficiency is rare in nature but a few Ca^{2+} deficiency disorders occur in horticultural crops such as 'tipburn', 'brown heart' in leafy vegetables, 'black heart' in celery, 'blossom end rot' in watermelons, tomato, pepper, 'fruit cracking' in tomato, 'bitter pit' in apples, 'empty pod' in peanuts and 'internal brown spot', 'hollow heart' and 'softrot' in potatoes. With rapid plant growth, the structural integrity of stems and the quality of fruit produced is strongly coupled to Ca^{2+} availability. Calcium being a universal second messenger acts as a mediator of stimulus-response coupling in the regulation of diverse cellular functions (Allen and Schroeder, 2001). Calcium was also reported to play a role in signal transduction leading to oxidative burst and plant defense (Miura et al., 1999). Calcium is also known to act as an activator of many enzymes like ATPase, phospholipases, amylase and succinate dehydrogenase. Experiments with

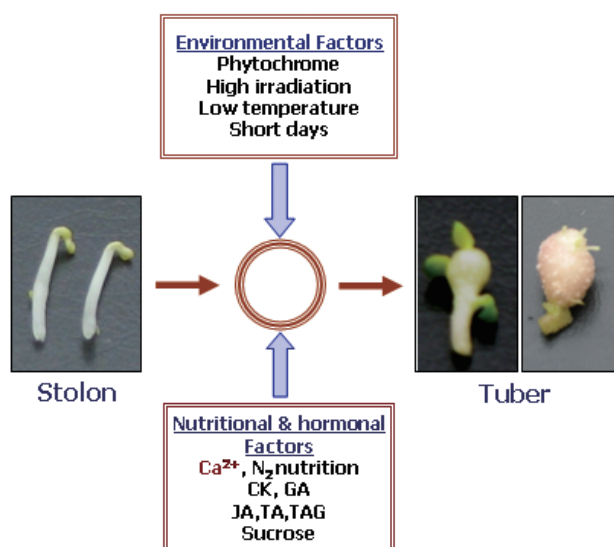


Figure 1. Interaction of various environmental and nutritional factors influencing tuber induction in potato. CaM, calmodulin; CK, cytokinin; GA, gibberellic acid; JA, jasmonic acid; TA, tuberonic acid; TAG, tuberonic acid glucoside.

Phaseolus vulgaris L. cv. Contender suggested that Ca²⁺ was associated with stomatal closure, decrease of hydraulic conductivity, sap flow, leaf specific dry weight, leaf K⁺ and Mg²⁺ concentrations, and inhibition of CO₂ assimilation (Cabot et al., 2009).

Cytosolic Ca²⁺ in plant cells is maintained at a concentration of 100 nM in the absence of a stimulus but in response to an external stimuli including light, touch, wind, gravity, hormones, abiotic and biotic stresses, the [Ca²⁺]_{cyt} concentration is rapidly elevated via an increased Ca²⁺ influx due to the release of Ca²⁺ by Ca²⁺ channels in endoplasmic reticulum (ER), plasma membrane and other cell organelles (Poovaiah and Reddy, 1993; Bush, 1995) and then quickly returns to the basal level by Ca²⁺ efflux by Ca²⁺/H⁺ antiports and Ca²⁺ pumps producing a Ca²⁺ spike (Evans et al., 2001; Reddy, 2001). The most common signaling pathway that increases [Ca²⁺]_{cyt} concentration is the phospholipase C pathway. Many cell surface receptors, including G protein-coupled receptors and receptor tyrosine kinases activate the phospholipase C (PLC) enzyme. The PLC hydrolyses the membrane phospholipid PIP₂ to form 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG), two classical second messengers. The DAG activates the protein kinase C enzyme, while IP₃ diffuses to the ER, binds to its receptor (IP₃ receptor), which is a Ca²⁺ channel, and thus releases Ca²⁺ from the ER. The stimulus-specific increases in [Ca²⁺]_{cyt} are called calcium signatures (Evans et al., 2001). Current evidences indicate that apart from IP₃, cyclic ADP ribose (cADPR) influence the activity of Ca²⁺ channels and play an important role in elevating [Ca²⁺]_{cyt} in plant cells. The transduction of a wide range of Ca²⁺ signals in to a diverse biochemical and morphological responses is very complex phenomena. A number of factors are likely to be involved in controlling the specificity of Ca²⁺ to a given response. It has been shown that a given signal may induce a different Ca²⁺ signature in different cell types (Kiegle et al., 2000). Moreover, temporal and spatial changes of Ca²⁺ together with the extent of its amplitude are likely to contribute significantly for achieving the specificity in eliciting appropriate physiological responses (Hepler, 1997; McAnish and Hetherington, 1998). It is also evident that different signals cause distinct spatial and temporal changes in Ca²⁺ and this Ca²⁺ signature is likely to be important in achieving the specificity (Trewavas and Malho, 1998; McAnish and Hetherington, 1998; Allen et al., 1999; Trewavas, 1999).

The increase in the [Ca²⁺]_{cyt} concentration leads to the activation of various Ca²⁺-sensor proteins that convert these signals into a wide variety of biochemical changes. There are four different Ca²⁺ sensors that exist in higher plants namely calmodulin (CaM), CaM-like and other EF-hand containing Ca²⁺-binding proteins (e.g. calcineurin B-like proteins), Ca²⁺-regulated protein kinases and Ca²⁺-binding proteins without EF-hand motifs. The Ca²⁺ binding EF-hand motif is a 29 amino acid helix-loop-helix structure resembling a hand having the index finger and thumb (the two helices) (Harmon, 2003). Binding of Ca²⁺ to these EF-hand modules was first demonstrated in Ca²⁺

buffer protein parvalbumin (Moncreif et al., 1990). The binding of Ca²⁺ results in a conformational change in the sensor molecule and exposes the hydrophobic pockets, which in turn facilitate interactions of the sensor protein with a variety of target proteins. This ultimately results in the modulation of sensor protein activity or its ability to interact with other proteins and modulate their function/activity. Calmodulin is a Ca²⁺-binding, multifunctional, regulatory protein that plays a very important role in Ca²⁺ signaling in higher plants and animals. Calcineurin is a Ca²⁺/CaM-regulated protein phosphatase that are reported to dephosphorylate many transcription factors (TFs) (Rao et al., 1997; Masuda et al., 1998). The CDPKs are a class of plant protein kinases that contain a kinase domain and a Ca²⁺-binding domain. The activity of CDPK is stimulated by Ca²⁺ suggesting that CDPKs may function in Ca²⁺-mediated signaling pathways. Plants also possess other classes of Ca²⁺ regulated protein kinase such as the Ca²⁺/CaM dependent kinase (CCaMK). The CCaMK, a multi-functional protein, is characterized by the presence of a kinase domain, an autoinhibitory domain, a CaM-binding domain and a neural visinin-like Ca²⁺-binding domain in a single polypeptide. These kinases are reported to play an important role in inhibition of autophosphorylation and enhancing substrate phosphorylation (Patil et al., 1995).

Calcium and Ca²⁺ sensor CaM regulate the expression of structural and regulatory genes by acting on TFs (Figure 2). The elevated Ca²⁺ in the nucleus may bind directly to TFs and modulates their activity; Ca²⁺-loaded CaM binds directly to promoter sequences and regulates gene expression, which implies that CaM functions as a TF; most commonly, the Ca²⁺/CaM complex interacts with TFs and modulates either their DNA-binding or transcriptional activities or the Ca²⁺/CaM complex indirectly regulates transcription by associating with the multi-component transcriptional machinery consisting of Ca²⁺/CaM complex, transcription factor-binding protein (TFBP) and TFs. The TFBP can function as a bridge between Ca²⁺/CaM and TFs. Finally, the Ca²⁺/CaM complex regulates gene expression by modulating the phosphorylation status of TFs. This indirect regulation is achieved by a CaM-binding protein kinase and a CaM-binding protein phosphatase (Kim et al., 2009). Although Ca²⁺ is implicated in regulating a number of fundamental cellular processes involving cytoplasmic streaming, thigmotropism, gravitropism, photomorphogenesis and stress responses, the molecular mechanisms by which Ca²⁺ controls these processes are not studied well.

INFLUENCE OF SUPPLEMENTAL Ca²⁺ ON POTATO TUBERIZATION

Although Ca²⁺ removal by potato is not large, the Ca²⁺ nutrition is critical in potato tuber growth and health. Calcium is directly taken up by tubers, and the roots attached to tubers and stolons along with the water uptake (Habib and Donnelly, 2002). Recent studies have provided evidence for the role of Ca²⁺ in potato tuberization by improving the tuber number and tuber yield (Ozgen et al.,

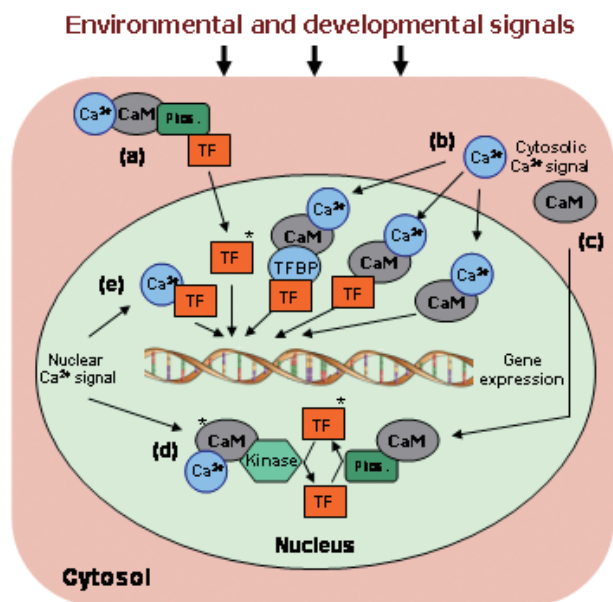


Figure 2. Schematic diagram showing the model of transcription regulation by Ca^{2+} /CaM in plants. (a) Cytosolic CaM responds to Ca^{2+} signals, activates a phosphatase, which dephosphorylates a transcription factor (TF). Consequently the TF is translocated to the nucleus and modulates transcription; (b) Cytosolic Ca^{2+} may transport to nucleus and effect gene expression by activating TF by binding with CAM through transcriptional factor binding protein (TFBP); (c) CaM responds to a cytosolic Ca^{2+} signal and is translocated into the nucleus, where it can affect the activity of phosphatases; (d) CaM responds to a nuclear Ca^{2+} signal and activates a CaM kinase, which phosphorylates a TF; (e) CaM responds to a nuclear Ca^{2+} signal and binds to a transcription factor and modulates gene expression.

2000, 2003; Ozgen and Palta, 2004; Ozgen et al., 2006; Chang et al., 2007). In a study conducted by Balamani et al. (1986), it was observed that tuberization in single node leaf cuttings of potato (*Solanum tuberosum* L.) was inhibited, when the explants were pre-treated with 5 mM ethyleneglycol-bis(4 (i-aminoethyl ether) N,N'-tetraacetic acid (EGTA, a Ca^{2+} chelator) and 50 mM Ca^{2+} ionophore (A23187), and the tuberization resumed when the explants were transferred to a CaCl_2 containing medium indicating the requirement of Ca^{2+} for potato tuberization. In a field study, the influence of Ca^{2+} application on potato tuber yield in four soil types was investigated (Simmons and Kelling, 1987). They have reported that application of 100 kg Ca ha^{-1} as $\text{Ca}(\text{NO}_3)_2$ in combination with CaSO_4 produced high quality tubers with increased tuber size. While, Ozgen et al. (2003) reported a decreased tuber number and increased tuber size with the application of Ca^{2+} indicating the influence of Ca^{2+} on tuberization signal. Their studies further suggested that soil Ca^{2+} influences tuberization by altering the hormonal balance at the stolon tip. Application of Ca^{2+} lead to increased localization of Ca^{2+} in tuber periderm (Simmons and Kelling, 1987; Ozgen et al., 2000; Ozgen and Palta, 2004) and the increased tuber Ca^{2+} con-

centrations inhibited the incidence of internal brown spot (IBS) in potato (*Solanum tuberosum* L.) (Ozgen et al., 2006; Chang et al., 2007). These studies also showed that Ca^{2+} fertilization has significantly increased total or marketable tuber yields suggesting the potential of Ca^{2+} nutrition for the production of high quality shipping potatoes. All these studies confirmed the important role of Ca^{2+} in regulating tuber Ca^{2+} content, tuber size and tuber yield.

ROLE OF Ca^{2+} -REGULATED PROTEINS IN POTATO TUBERIZATION

Calcium-dependent modulation of various cellular processes is mediated through intracellular Ca^{2+} -binding or Ca^{2+} modulator proteins, also known as Ca^{2+} -sensors. These proteins play an important role in decoding and transducing Ca^{2+} signals to activate specific targets in biological pathways. The four types of Ca^{2+} -sensor proteins found in plants can be divided into two major classes. The first class of sensors (called 'sensor relays', e.g. CaM and calcineurin B-like proteins without any responder domains) bind to Ca^{2+} and undergo conformational changes and in turn regulate the activity or function of a variety of other target proteins. The second class of sensors, called 'responders' possesses other effector domains (e.g. protein kinase or phospholipase domain) through which they relay the message to their down-stream targets. Though few evidences suggested the involvement of these proteins in tuberization, the exact mechanism of their action is not clear. Here, we summarize the major Ca^{2+} -regulator proteins and ion channels and their involvement in potato tuberization. The list of Ca^{2+} /CaM binding proteins, for which there are evidences suggesting their regulatory role in plant metabolic processes associated with tuberization, is summarized in Table 1.

1. Calmodulin (CaM), a Ca^{2+} -sensor protein

Calmodulin is a small molecular weight acidic protein composed of approximately 148 amino acids with four EF-hand motifs that bind to four Ca^{2+} ions. It is a multifunctional, regulatory protein that plays an important role in Ca^{2+} signaling in higher plants and animals. Calcium in association with its sensor protein, CaM has been shown to affect a number of metabolic processes in plants (Trewavas and Malho, 1997; McAnish and Hetherington, 1998). Calmodulin has no catalytic activity of its own but upon binding with Ca^{2+} ; it binds to and activates numerous target proteins involved in a variety of cellular processes with the exception of myosins in animals which bind to CaM in absence of Ca^{2+} . Binding of Ca^{2+} to CaM exposes two hydrophobic surfaces surrounded by negative charges, one in each globular domain. The Ca^{2+} /CaM complex then bind to its targets through hydrophobic and electrostatic interactions with long hydrophobic side chains in the target sites (Snedden and Fromm, 1998; Hoefflich and Ikura, 2002). The Ca^{2+} /CaM modulates the activity of Ca^{2+} /CaM-binding proteins through mechanisms such as 'relieving

Table 1. Ca²⁺/CaM induced signal networks controlling various plant processes associated with tuberization.

Ca ²⁺ /Ca ²⁺ sensor	Effector protein	Metabolic change	Function	Reference
Ca ²⁺	-	Altered GA levels	Cell division and α -amylase secretion during germination in barley	Bush and Jones, 1988; Gilroy and Jones, 1992
Ca ²⁺	CBL9-	-	Sucrose metabolism in mungbean; starch synthesis in soybean	Nakai et al., 1998; Zhang and Chollet, 1997
CaM	-	GA morphotype	Tuber induction	Poovaiah et al., 1996
Ca ²⁺ /CaM	NtER1	Ethylene signaling	Plant senescence	Yang and Poovaiah, 2000
Ca ²⁺	CDPK	ACC synthase activity	Reduced ethylene levels in maize	Sebastia et al., 2004
Ca ²⁺	CBL9	ABA signaling	Seed germination in <i>Arabidopsis</i>	Pandey et al., 2004
CaM	KCBP	Interaction with microtubules	Cell division	Song et al., 1997; Narasimhulu and Reddy, 1998
SCaM4, SCaM5	-	SA related gene expression	Fungal resistance	Heo et al., 1999
Ca ²⁺ /CaM	NAD kinase	-	Oxidative burst	Muto, 1982; Liao et al., 1996; Harding et al., 1997
CaM	CuZnSOD	Altered GA metabolism	Defense against oxidative burst, tuber induction	Kim et al., 2007
CDPK	-	-	Tuber development	MacIntosh et al., 1996
<i>NtCDPK</i>	-	-	Sucrose metabolism, phosphorylation of membrane proteins	Iwata et al., 1998; Yoon et al., 1999
CDPK	-	Reduced GA levels	Tuber induction in potato	Gargantini et al., 2009
Ca ²⁺	Aquaporins	-	In flowering and in cell expansion during fruit development in wild potato	O'Brien et al., 2002

CaM, calmodulin; Ca²⁺/CaM, Ca²⁺-bound CaM; CBL, calcineurin B-like proteins; CCaMK, chimeric Ca²⁺/CaM-dependent protein kinase; CDPK, Ca²⁺ dependent protein kinase; GA, gibberellic acid; KCBP, Kinesin like CaM-binding protein; SA, salicylic acid; SOD, superoxide dismutase; TFs, Transcription factor; ZBF3, Z-box binding factor.

auto inhibition' (Chin and Means, 2000), 'active site remodeling' (Drum et al., 2002) or 'dimerization of channel proteins' (Schumacher et al., 2001) (Figure 3). Through one of these mechanisms, Ca²⁺/CaM activate numerous target proteins involved in a variety of cellular processes (Figure 4). CaM-binding proteins have roles in regulation of plant metabolism, cytoskeleton function, phytohormone signaling, ion transport, protein folding, protein phosphorylation and dephosphorylation (Snedden and Fromm, 1998; Yang and Poovaiah, 2000; Bouche et al., 2005). Recent reports suggested that CaM also participates indirectly in the regulation of gene expression by acting through a CaM-binding protein kinase and a CaM-binding protein phosphatase (Liu et al., 2007, 2008).

There are evidences to indicate the involvement of Ca²⁺/CaM at some stage downstream in the tuber induction pathway. Tuberization was inhibited in single leaf cuttings of potato cv. Russet Burbank by the addition of CaM antagonists such as chlorpromazine and trifluoperazine to the liquid culture medium and tuberization resumed when the cuttings were transferred to CaCl₂ medium (Balamani et al., 1986). In another study, Jena et al. (1989)

demonstrated an increased expression of CaM mRNA in the stolon tip and suggested the signaling roles of Ca²⁺ and CaM in tuber induction. Multiple calmodulin isoforms have been reported in *Arabidopsis* (Lee et al., 1995), soybean (Liao et al., 1996) and potato (Takezawa et al., 1995) showing differential expression during plant growth and tuber development. It has also been hypothesized that depending on the relative abundance of a specific isoform a given Ca²⁺ signal might produce different biochemical consequences by virtue of its CaM isoform-dependent selective activation/inhibition of particular target enzymes. Out of the eight isoforms of CaM in potato, the expression levels of PCM1, 5 and 8 were highest in stolon tip and decreased with tuber development (Takezawa et al., 1995). In addition, enhanced expression of *PCMI* promoter was observed in the stolon tip and the deduced amino acid sequence of *PCMI* had several unique substitutions, especially in the fourth Ca²⁺-binding area. The expression of *PCM6* did not vary much in the tissues tested, except in the leaves, where the expression was lower; whereas, the expression of *PCM4* was very low in all the tissues. The expression of *PCM2* and *PCM3* was not detected in any of

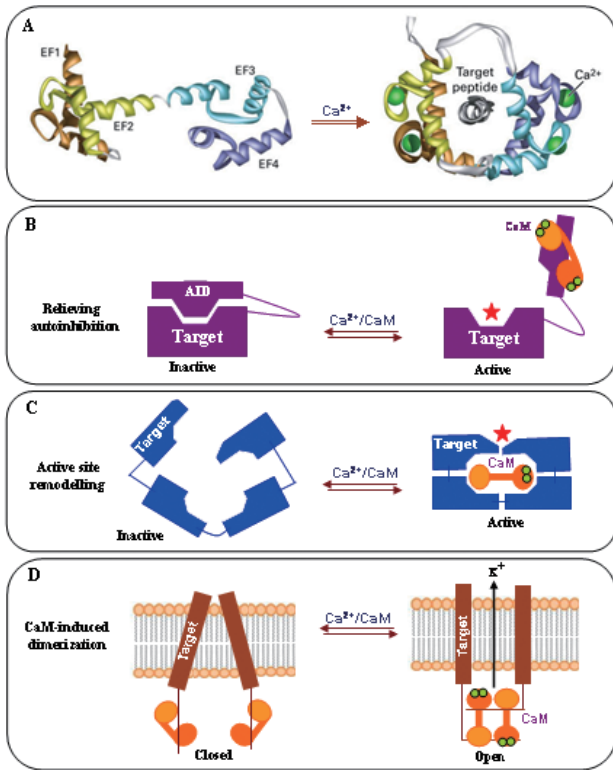


Figure 3. Calmodulin structure and its activation mechanisms. (A) Crystal structure of CaM with 4 EF hand motifs that can bind to 4 Ca²⁺ ions. Binding of 4 Ca²⁺ allows CaM to bind target proteins. (B-D) Protein activation mechanisms by calmodulin (B) Relieving autoinhibition: Calmodulin binding to the target induces a conformational rearrangement that displaces the auto-inhibitory domain (AID) and allows for full enzyme activity. (C) Active site remodeling: upon Ca²⁺/CaM binding, a helical domain of oedema factor undergoes a rotation away from the catalytic core, which stabilizes a disordered loop and leads to enzyme activation (D) CaM-induced dimerization: two CaM molecules interact with two K⁺ channel domains of a K⁺ channel upon Ca²⁺-binding. The concept is taken from Hoeflich and Ikura (2002).

the tissues tested. Among these genes, only *PCMI* showed increased expression following touch stimulation (Takezawa et al., 1995). Transgenic potato plants over-expressing the isoform *PCM1* were found to be inhibited in their tuberization response (Poovaiah et al., 1996). Transgenic plants showing a moderate increase in *PCMI* mRNA levels exhibited strong apical dominance, produced elongated tubers, and were taller than the controls (Poovaiah et al., 1996). Surprisingly, the plants expressing the highest level of *PCMI* mRNA did not form underground tubers. Instead, these plants produced aerial tubers when allowed to grow for longer periods. In addition to reduced tuber induction, the transgenic plants exhibited a phenotype reminiscent of gibberellic acid (GA)-treated plants. These results showed differential influence of CaM isoforms on potato tuberization indicating a particular isoform(s) is actively involved in signal transduction events leading to potato tuberization.

Transgenic soybean plants expressing CaM isoforms SCaM4 and SCaM5 showed constitutive expression of salicylic acid (SA) related genes and enhanced disease resistance to fungal pathogens and tobacco mosaic virus (Heo et al., 1999). The inductive role of SA and JA in potato tuberization is well documented (Pelacho and Mingo-Castel, 1991; Koda, 1992). These compounds may promote tuberization by antagonizing the inhibitory effects of GA (Jackson, 1999). Yang and Poovaiah (2000) reported the involvement of Ca²⁺/CaM in ethylene signal transduction and senescence also. Earlier, ethylene inhibited tuberization in potato by eliminating or overriding the action of cytokinins and cell division (Dimalla and Van Staden, 1977) while Vreugdenhil and van Dijk (1989) reported a dual role of ethylene and ethylene precursors on potato tuberization. The CaM also reported to influence NAD kinase (Muto, 1982; Liao et al., 1996) and involved in NADPH-dependent oxidative burst (Harding et al., 1997). Experiments with lily copper zinc superoxide dismutase (chCuZnSOD) in potato suggested the role of oxidative burst in tuberization through affecting the intrinsic GA levels (Kim et al., 2007). Oxidative stress caused by the production of H₂O₂ also elicited the production of SA and ethylene in transgenic tobacco plants (Chamngopol et al., 1998). The two key enzymes in brassinosteroid biosynthesis namely DWF1 (gene identified in *Arabidopsis* DWARF1 mutant), CPD (encoding a steroidogenic cytochrome P450) were found to be Ca²⁺/CaM dependent and it has been suggested that Ca²⁺/CaM binding is critical for their expression especially DWARF1 function in brassinosteroid biosynthesis (Du and Poovaiah, 2005). The role of Ca²⁺/CaM signaling in brassinosteroid biosynthesis and potato tuberization is worth investigating.

A Ca²⁺-dependent plant-specific CaM-binding nuclear protein called potato CaM-binding protein (PCBP) was identified by screening an expression library prepared from developing potato tubers (Reddy et al., 2002).

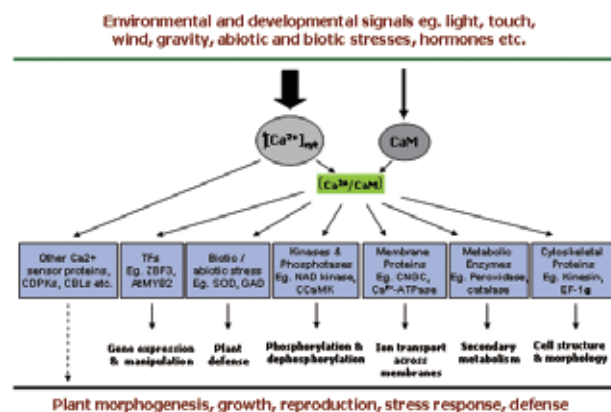


Figure 4. The Ca²⁺/CaM-mediated signaling networks in plants. Different environmental and developmental signals trigger changes cytosolic Ca²⁺ signals. The Ca²⁺ signatures are decoded by Ca²⁺ sensors, CaM, CDPK and CBL. The activated Ca²⁺/CaM complex binds to numerous target proteins and modulates their activities in various cell physiological responses.

Though it was isolated from a swelling stolon tip library, its expression was seen in all the tested tissues of potato including vegetative and reproductive parts. The binding of PCBP with CaM in a Ca²⁺-dependent manner indicates its involvement in a Ca²⁺ signaling pathway and likely regulation of its activity/ function by Ca²⁺. A potato CaM binding protein, a homolog of mammalian multidrug-resistant P-glycoprotein (PMDR1) was isolated from a stolon tip library and the *PMDR1* mRNA was constitutively expressed in all organs studied with higher expression in the stem and stolon tip (Wang et al., 1996). Further, the *PMDR1* mRNA expression was highest during tuber initiation and decreased during tuber development suggesting its possible role in tuber induction. Another protein, CaM-binding kinesin protein (KCBP) was found to be involved in cell division (Song et al., 1997; Narasimhulu and Reddy, 1998). The role of cell division may contribute to the active growth of tuber during tuber development in potato (Palmer and Smith, 1969; Smith and Palmer, 1970; Van Staden and Dimalla, 1976, 1977; Menzel, 1985).

2. Calcium dependent protein kinases (CDPKs)

The calcium dependent protein kinases are another group specialized Ca²⁺-modulated proteins that serve as receptors for Ca²⁺ signals. These are serine-threonine protein kinase with a CaM-like domain at the C-terminal region. Five CDPK/SnRK subfamilies namely CDPK, CCaMK (Ca²⁺ or Ca²⁺/CaM-regulated kinases), CaMK (CaM-dependent protein kinases), CRK (CDPK related kinases) and CIPKs (CBL-interacting protein kinases) are known to be regulated either directly or indirectly by cytosolic Ca²⁺. Among these, two subfamilies (CDPK and CCaMK) contain EF-hands at their C-terminal domain; three subfamilies (CCaMK, CaMK and CRK) bind to CaM and one subfamily (CIPK) binds to CBLs. The CDPKs are encoded by multigene families, for example in *Arabidopsis thaliana* there are 34 genes for CDPKs (Hrabak et al., 2003). Southern blot analysis suggested the occurrence of more than one CDPK isoforms in potato, which were developmentally regulated (Raices et al., 2001). They also reported a correlation between the increase in CDPK activity, Ca²⁺-dependent phosphorylation and the morphological changes associated with the tuber development.

A Ca²⁺-dependent protein kinase from *Solanum tuberosum* L., called StCDPK1, with a highly conserved myristoylation site, was reported to be expressed in tuberizing stolons and sprouting tubers (Raices et al., 2001). The StCDPK1 was suggested to trigger a cascade of phosphorylation events during tuber induction and involved in the events leading to tuber formation (MacIntosh et al., 1996). These reports indicate that protein phosphorylation mediated by protein kinases is important for transcriptional activation of some of the tuberization genes in potato. Further, *StCDPK1* expression is positively modulated by sucrose application (Raices et al., 2003) and tuberization-promoting phytohormones, abscisic acid (ABA) and 6-benzyl adenine (BA) (Gargantini et al., 2009). However,

transgenic potato lines ($\beta 7$) with reduced expression of *StCDPK1* showed early tuberization when grown under tuber inducing conditions (continuous dark and 20°C temperature) without the addition of CCC (inhibitor of GA) and developed more tubers than control in the presence of hormones that promote tuberization (ABA and BA) (Gargantini et al., 2009). They have also reported that GA treatments enhanced *StCDPK1* expression and under *in vitro* conditions $\beta 7$ plants tuberized earlier and upon exposure to GAs they formed shorter stolons than wild types. These results with $\beta 7$ line suggested that StCDPK1 could be a positive regulator of elongation response and a negative regulator of tuberization. The role of StCDPK1 in potato tuberization is also evident by its enhanced expression under increasing sucrose concentrations (4-8%) and phytohormones in control potato plants (Gargantini et al., 2009), which suggests that StCDPK1 could be a target of ABA action with a synergistic effect of sugar and ABA on the kinase expression. All these results along with the insensitivity of $\beta 7$ lines to GA action suggest an important role of StCDPK1 in GA-signaling forming a converging point for the inhibitory and promoting signals that influence the onset of potato tuberization.

The treatment of potato plants with JA resulted in reduced mRNA levels for *StCPK2* (Ulloa et al., 2002). Similarly, tobacco *NtCDPK1* gene was found to be transcriptionally regulated by phytohormones (ABA, GA and cytokinin), Ca²⁺, methyl jasmonate, wounding, fungal elicitors, chitosan and NaCl in leaves (Yoon et al., 1999). Studies also suggested the regulation of CDPK activity by exogenous ABA during cold-stress responses in rice (Komatsu et al., 2001). Further, ABA-stimulated *de novo* synthesis of ACPK1 implicates its role in regulating a development related ABA-signaling (Yu et al., 2006). In another study, CDPK was reported to phosphorylate the enzyme ACC synthase, which catalyses the biosynthesis of ethylene in maize (Sebastia et al., 2004). Similar influence of CDPK on ethylene during potato tuberization can not be ruled out.

Iwata et al. (1998) characterized sucrose inducible CDPK isoform from tobacco leaves and suggested its possible role in sucrose metabolism. Enhanced expression of *StCDPK1* and other tuber-specific genes during *in vitro* culturing of potato on high sucrose or high sorbitol containing medium suggests the sugar regulation on *in vitro* tuber formation (Garner and Blake, 1989). Also, *StCDPK1* is reported to be a key mediator in sucrose-signaling pathways during tuber induction and development (Raices et al., 2003). In addition the sucrose inducible transcription of *StCDPK1* is blocked by phosphatase inhibitors (okadaic acid) suggesting that dephosphorylation events mediated by protein phosphatases modulate *StCDPK1* expression. MacIntosh et al. (1996) reported that potato CDPK was strictly dependent on Ca²⁺ for its activation. They reported that CDPK activity was 2.5 - 3 times higher at early stages of tuberization than in non-tuberized plants and was reduced to one-half of its original activity as the tuber ma-

tures. In the early stages of tuberization, Ca^{2+} -dependent phosphorylation of endogenous targets was observed and the phosphorylation of majority of polypeptides was increased in the presence of Ca^{2+} suggesting the stimulatory role of Ca^{2+} on protein kinase activity. These polypeptides were not labeled in non-tuberizing plants or in completely formed tubers, indicating that this phosphorylation is a stage-specific event. The enhanced activity of protein kinase during early stages of potato tuberization indicates its role in signal transduction triggering tuber formation (Ulloa et al., 1997). Although CDPKs have been implicated to act as key regulators of many signaling pathways, very little is known about which particular CDPK acts as the calcium sensor in each case. The major challenge in the future will be to elucidate which CDPK isoform functions in and interacts with which pathway with reference to potato tuberization. It is believed that CDPK mediates oxidative burst induced by $[\text{Ca}^{2+}]_{\text{cyt}}$ and is involved in the activation process of NADPH oxidase (Grant et al., 2000; Xing et al., 2001). Thus the role of oxidative burst mediated by Ca^{2+} and CDPK during potato tuberization needs to be investigated.

3. Other Ca^{2+} -binding proteins with EF hands

In addition to CaM, few recent studies indicate the presence of numerous CaM-like proteins in plants. However, the function of these proteins in Ca^{2+} signaling is not fully characterized. These CaM-like proteins differ from the CaM as they contain more than 148 amino acids, and have one to six EF hand motifs with limited homology to CaM (Snedden and Fromm, 1998). Hence, these proteins may be functionally distinct from CaM and involved in different Ca^{2+} -mediated cellular functions. Recently, one such family of Ca^{2+} binding proteins called calcineurin B-like proteins (CBL) has been identified in this group. Calcineurin is a Ca^{2+} /CaM-regulated protein phosphatase that dephosphorylates transcriptional factors such as NAFT (nuclear factor of activated T cells) (Rao et al., 1997; Masuda et al., 1998). The calcineurin B-like proteins possess three EF hands and they interact and regulate the function of a group of protein kinases called CBL interacting protein kinases (CIPKs) (Batistic and Kudla, 2004; Kolukisaoglu et al., 2004). The CBLs were also reported to play a role in abiotic stress response pathways (Albrecht et al., 2003; Cheong et al., 2003). Among them, CBL9 is specifically involved in mediating ABA signaling during *Arabidopsis* seed germination (Pandey et al., 2004). The negative influence of CBL9 on ABA inhibition of seed germination further confirms the ABA and GA antagonism. It was earlier reported that ABA promotes longitudinal arrays of microtubules and reverses the effect of GA_3 on microtubule orientation (Shiboaka, 1993). The positive influence of ABA on tuber induction in potato was also documented earlier (Xu et al., 1998). Thus the promotive effects of ABA on tuberization also appear to be due to the antagonistic effects of ABA and GA. The CBL9 is also reported to play a role in sucrose metabolism and starch synthesis in soybean (Zhang and Chollet, 1997; Na-

kai et al., 1998). Sucrose is known to positively influence tuberization in potato and sucrose to starch conversion is a key metabolic event during tuber development. Similarly, the role of CBL9 in tuberization through altered sucrose metabolism may be expected.

4. Other Ca^{2+} -binding proteins without EF hands

There are several proteins that bind Ca^{2+} but do not contain EF-hand motifs, e.g. annexins, calcitriculin, phospholipase D and pistil expressed Ca^{2+} -binding protein (Reddy, 2001). Although the exact function of annexin is not known, the plant annexins are implicated in secretory processes and some have ATPase, peroxidase or F-actin binding activities (Lim et al., 1998). They were also implicated in Ca^{2+} signaling during root nodulation, pathogen attack, ABA response, fruit ripening and cold acclimation (White, 2001). Calcitriculin is a Ca^{2+} sequestering protein in the ER and functions as a chaperone (Baluska et al., 1999). The activity of phospholipase D is implicated in cellular responses to ethylene, ABA, α -amylase synthesis in aleurone cells, stomatal closure, pathogen responses, leaf senescence and drought responses (Ritchie et al., 2002). Similar influence of phospholipase D on ethylene and ABA signaling during potato tuberization is worth investigating to reveal the role of phospholipase D in Ca^{2+} signaling. Other Ca^{2+} -binding proteins include calsequestrin and calnexin, which are involved in Ca^{2+} homeostasis, protein folding and post-translational modifications (Michalak et al., 1998). The role of these of proteins in potato tuberization is not known.

5. Aquaporins

Aquaporins are proteins embedded in the cell membrane that regulate the flow of water. Aquaporins, also known as water channels selectively conduct water molecules in and out of the cell, while preventing the passage of ions and other solutes. In storage roots of *Beta vulgaris*, $[\text{Ca}^{2+}]_{\text{cyt}}$ has been shown to up- and down-regulate the water channel activity *vis-a-vis* aquaporin gating (Alleva et al., 2006). Studies with transgenic tobacco plants overexpressing a potato plasmalemma aquaporin encoding gene *StPIP1* showed that aquaporins were involved in rapid water transport under drought conditions, root development, seed germination and seedling growth (Wu et al., 2009). Johansson et al. (1996, 1998) have reported an influence of Ca^{2+} ions on aquaporins. When the water potential of the apoplast is high, plasma membrane aquaporins are opened by phosphorylation mechanisms mediated by a Ca^{2+} -dependent protein kinase. Experiments with wild potato *Solanum chacoense* Bitt. indicated the role of plasma membrane aquaporin in potato flowering and in cell expansion during fruit maturation and development (O'Brien et al., 2002). A similar interplay between $[\text{Ca}^{2+}]_{\text{cyt}}$ and water channel activity is worth investigating in potato tuberization.

In addition to plant growth processes and hormonal responses, Ca^{2+} is known to regulate plant sensing to en-

environmental factors like light and photoperiod (Sanders et al., 1999). Evidence showing the involvement of Phytochrome B (PHYB) in photoperiod control of potato tuberization was reported (Jackson et al., 1996). The PHYB affected tuberization in the PHYB-deficient antisense plants of potato through influencing the GA biosynthetic pathway (Davies et al., 1986; Olsen et al., 1995). Photoperiod is also known to affect the production and export of sucrose and another signaling molecule in tuberization. The possibility of the role of an oxidative burst caused by Ca²⁺ ions in the signaling network of the tuberization process cannot be ignored. Oxidative burst caused by abiotic stress in crop plants, results in the generation of reactive oxygen species (ROS) and is known to induce an intracellular signaling pathway (Grant et al., 2000). Transgenic potato plants down-regulated for CuZnSOD have shown to accumulate higher levels of superoxides, which were found to affect the expression of GA biosynthetic enzymes leading to reduced the GA levels (Kim et al., 2007). Reduced GA levels in leaves and shoots induced spontaneous tuberization in down-regulated plants confirming the inhibitory role of GA in potato tuberization. Tuberization in potato is characterized by an enhanced activity of lipoxygenase (LOX) (Kolomiets et al., 2001; Nam et al., 2005). Preliminary studies on the influence of Ca²⁺ on *in vitro* tuberization of potato in our laboratory suggested Ca²⁺ had affected the activity of LOX indicating its indirect influence on tuberization proteins through some unknown mechanism (unpublished result). The LOX catalyzes lipid peroxidation by using membrane

lipid components, particularly unsaturated fatty acids as substrates, and its activity is highly correlated with the cellular redox state during oxidative burst caused by hydrogen peroxide (H₂O₂) (Maccarrone et al., 2000). Thus, the possibility of a cross-talk with an oxidative burst-mediated redox signaling pathway during tuberization should be considered. Though Ca²⁺ and its sensor proteins, CaM, CDPKs and CBLs are known to influence a number of biological processes in potato, the exact role of each sensor protein in the signaling mechanism of potato tuberization is not clear. The predicted possible molecular mechanisms of Ca²⁺/CaM-mediated signal pathways controlling tuberization in potato is depicted in Figure 5. In summary, signaling cascades in potato tuberization are yet to be elucidated completely by molecular and biochemical characterization of the individual components.

CONCLUSIONS AND FUTURE PERSPECTIVES

In recent years, Ca²⁺ signaling has received a great attention because of its involvement in many plant developmental processes like growth, reproduction, biotic and abiotic stress responses. Studies also suggested the influence of Ca²⁺ in potato tuberization under *ex vitro* conditions. Accumulating evidences indicated that Ca²⁺ influences tuberization through Ca²⁺ modulator proteins, CaM, CDPK, CBLs and channel proteins. Some of these proteins have been shown enhanced expression in stolon tips and developing tubers and they were reported to be involved in signal transduction pathways that regulate tuberization in potato. Calcium is also involved in ABA and ethylene signaling during abiotic stress tolerance in plants. A similar involvement of Ca²⁺ signaling in ABA and ethylene metabolism during potato tuberization needs to be investigated. Further, the influence of Ca²⁺/CaM on brassinosteroid biosynthetic enzymes suggests the possible role of Ca²⁺ signaling in brassinosteroid-regulated tuber induction in potato. Although, last few years have seen considerable advances in the understanding of the molecular and biochemical processes underlying potato tuber development, the exact signal transduction pathways induced by Ca²⁺ need to be investigated. Further studies in this area involving the characterization of Ca²⁺-sensors and their target proteins will elicit the clues for Ca²⁺-mediated signaling pathways in potato tuberization.

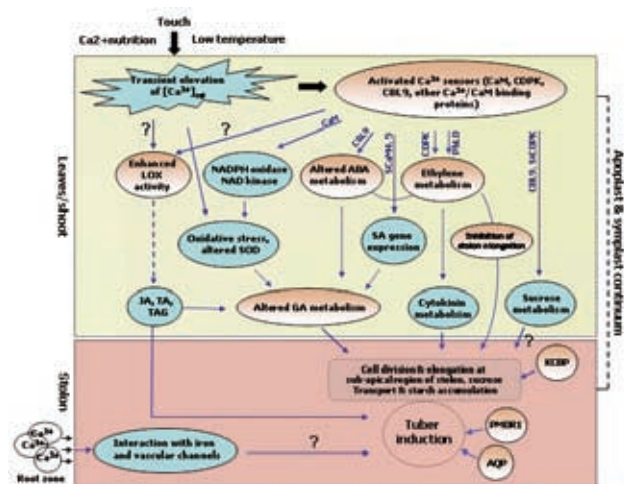


Figure 5. Possible mechanisms of Ca²⁺-mediated signal pathways controlling potato tuberization. Solid block arrow inductive path, thick arrow transduction pathway, thick dot arrow biosynthetic pathway, thin dot arrow interactive response, thick dash line apoplastic/symplastic continuum. ABA, abscisic acid; AQP, aquaporin; CaM, calmodulin; CBL, calcineurin B-like proteins; CDPK, Ca²⁺ dependent protein kinase; CK, cytokinin; GA, gibberellic acid; JA, jasmonic acid; KCBP, kinesin like CaM-binding protein; LOX, lipoxygenase; PhLD, phospholipase D; PMDR1, homolog of multidrug-resistant P-glycoprotein; SA, salicylic acid; SOD, superoxide dismutase; TA, tuberonic acid; TAG, tuberonic acid glucoside.

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