**Role of Calcium** S**ignalling in Plant Defense Response against Diseases**

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**INTRODUCTION**

Calcium is an essential signalling macronutrient in plant cells. Plants are constantly facing various biotic stresses such as fungi, bacteria and viruses and abiotic stresses like changes in temperature, high salinity, cold and drought. In unstimulated cells, cytosolic Ca2+ concentrations are usually maintained at lower levels 100 nM through the activity of Ca2+-ATPases and Ca2+/H antiporters in cell membranes (Bush, 1995: Sanders’ *et al* 1999). Perception of biotic and abiotic stresses often leads to increase in cytosolic free Ca2+ concentration in plant cells to activate signalling responses for decoding internal and external stimuli, translating them into physiological and gene expression responses. Increase in cytosolic Ca2+ is mediated by several families of calcium permiable ion channels, including cyclic nucleotide-gated channels (CNGCs), ionotropic glutamate receptors, two-pore channel 1 (TPC1), annexins and several types of mechanosensitive channels, these channels are predominantly involved in cell signalling. Calcium-permeable channels have been recorded in the plasma membrane, tonoplast, endoplasmic reticulum, chloroplast and nuclear membranes of plant cells that mediate calcium influx into cytosol. The specificity of the calcium signal to produce appropriate defense response is thought to be encoded by different amplitude, temporal or spatial changes in cytoplasmic calcium concentration (Trewavas, 1999: Malho, 1998: McAinsh, 1998). Elevations in free calcium concentration in plant cells is an essential early events following the perception of different environmental stimuli. Alterations in the Ca2+ concentrations are sensed by calcium- modulated proteins or calcium sensors including calmodulin, calcium-dependent protein kinases, calmodulin-like proteins (CMLs), calcium and calcineurin B-like (CBLs) proteins, which decode the encoded calcium signals into specific cellular and physiological responses in order to survive environmental challenges. Among calcium sensors kinases represent ‘responders’ and are capable of directly transducing a signal via catalytic activity, whereas CaMs/CMLs and CBLs are non-catalytic relay sensors that regulate dowstream targets. Calcium binding to Ca2+ sensors will induce a conformational change allow them to interact with downstream effectors or a direct stimulation of the kinase activity (Harmon et al., 2000). The diversity of Ca2+ sensors and their downstream targets contributes to a second layer of specificity, allowing the transduction of various primary stimuli into distinct biological responses (Hashimoto and Kudla, 2011).

**Characteristics of Calcium Ion Permeable Channels**

**Cyclic Nucleotide-Gated Channels (CNGCS)**

The cyclic nucleotides cAMP and cGMP are ubiquitous molecules that play key roles in the regulation of diverse cellular processes, gene expression and signal transduction (Newton RP and Smith CJ, 2004: Trewavas et al., 2002). Cyclic nucleotide gated ion channels (CNGCs) provide a pathway for Ca2+ conductance across the plasma membrane of plant cells and facilitate elevation in cytosolic Ca2+ in response to biotic and abiotic stimuli that results in cyclic nucleotide production and the activation of CNGCs, which leads to downstream generation of pivotal signaling molecules such as nitric oxide (NO). Pathogen recognition by a receptor leads to the activation of nucleotidyl cyclase in plant cell that give rise to cyclic nucleotide concentration. The increase in the cyclic nucleotide concentration results in the activation of the cyclic nucleotide gated ion channel (CNGC) and cytosolic Ca2+ elevation. Ca2+ elevation increases the amount of Ca2+ bound to calcium sensors that regulates the synthesis of downstream signaling components nitric oxide (NO) and hydrogen peroxide (H2O2), which are essential for the development of the hypersensitive response (HR). The increased amounts of calcium in the cytosol also compete with cyclic nucleotide for binding to the CNGC, blocking further Ca2+ conductance by the channel. It is found that CNGCs participate in heat stress signaling in *Arabidopsis* and *Physcomitrella* *patens* provide a mechanistic basis for the well-documented heat-induced cytoplasmic calcium elevation (Finka et al., 2012; Gao et al., 2012). Heat stress raises cAMP in Arabidopsis and activates a root cell plasma membrane HACC that is also activated by membrane-permeant cAMP (Gao et al., 2012). This HACC is absent from the Atcngc6 mutant, leading to aberrant heat shock protein expression and lowered thermotolerance. Most of the CNGCs are expressed at the Plasmic membrane. They are abundant in the root and leaf epidermis where they probably act for perceiving and encoding environmental stimuli. CNGCs are found in guard cells and the mesophyll, where they take part in the control of stomata closure and photosynthesis (Gobert et al., 2006; Jammes et al., 2011). CNGCs are also responsible for the nuclear Ca2+ oscillations in the symbiotic signalling pathway in legume roots; they mediate a targeted nuclear release of the ER Ca2+ store (Charpentier et al., 2016). PTI is also regulated by one of the calcium channel in Rice (OsCNGC9) which acts as a Ca2+ permeable divalent cation-selective inward channel and is activated by OsRLCK185-mediated phosphorylation (Wang et al, 2019).

**Ionotropic Glutamate Receptors**

Glutamate receptors (GLRs) are non-selective cation channels that regulates Ca2+ influx in several species of higher plants in both dicotyledons and monocotyledons. In plants 20 genes encode for GLRs that are differentially activated by Glu and Gly, as well as by other amino acids, and mediate an increase of cytosolic Ca2+ (Chiu et al., 2002; Qi et al., 2006; Stephens et al., 2008). GLRs are important for plant Ca2+ nutrition (Kim et al., 2001; Demidchik and Maathuis, 2007) but also in mediating Ca2+ responses upon cold stress (Meyerhoff. et al., 2005) or excess aluminum (Sivaguru et al., 2003). Overexpression of another GLR, At GLR3.1, impaired long-term stomatal closure but did not affect the short-term stomatal closure response or the kinetics of Ca2+ oscillations that were imposed by extracellular Ca2+ (Cho et al., 2009). GLR proteins are anticipated to have three transmembrane domain structures: a pore-forming domain, and two putative ligand binding hand motifs with preferential expression in root tissues (Price et al., 2013). Some specific subunits are expressed in leaf mesophyll, guard cells and pollen tubes (Weiland et al., 2015). Michard et al. (2011) demonstrated that GLR1.2 is expressed in pollen tubes and is practically involved in the polar Ca2+ influx required for pollen tube growth and elongation. Knocking out GLRs afflicted with metal- ion homeostasis, pollen incompatibility, immunity and photosynthesis (Weiland et al., 2015). Exogenous Glutamate causes an accumulation of extracellular ATP in Arabidopsis roots (Dark et al., 2011) that, in turn, can activate plasma membrane Ca2+ influx channels via ROS production by a NADPH oxidase (Demidchik et al., 2009).

**Two-Pore Channel 1 (TPC1):**

Voltage-gated organellar cation channel in vacuolar membrane in Charophyte algae and in all terrestrial plants and requires both voltage and cytosolic calcium concentration for activation. TPC1 has a single gene in A. thaliana (Peiter et al., 2005) with six transmembrane domain (6-TM) structure having several canonical Ca2+-binding sites (EF-hands) and are activated by cytosolic Ca2+ and its relative expression determines the vacuolar Ca2+ storage capacity (Gilliham et al., 2011) . A pre-requisite for TPC1 opening is Ca2+ binding to the cytosolic EF-hand (Guo et al., 2016; Kintzer & Stroud, 2016). TPC1 channels are essential components in long-distance Ca2+ signalling. These channels play crucial role for systemic Ca2+ response to herbivory in leaves (Kiep et al., 2015) and for rapid Ca2+ signal propagation along the root in response to local NaCl application (Choi et al., 2014). A rapid Ca2+wave in the latter case also requires a ROS-Ca2+ hub in the PM, in addition to TPC1 (Evans et al., 2016). These channels have also reported as the most likely oxygen modulators operating in plants under conditions of soil flooding (Wang et al., 2017). TPC1s have been identified as a pathway for Ca2+ entry across the tonoplast in response to cold shock, sucrose,, salicylic acid, as well as elicitors. Changes in the cytosolic Ca2+ levels of plant cells in response to pathogen exposure have been observed and recognized as a vital early event for plant defense responses like Salt stress-induced Ca2+ROS waves. ROS are produced in RBOHD and diffuse through the apoplast, activating ROS-sensitive Ca2+ channels in the plasma membrane. These channels release Ca2+ into the cytosol that activate TPC1 proteins which, directly or indirectly, mediate Ca2+ release from the vacuole. Combined, this Ca2+ activates further RBOHD proteins, giving rise to a self-propagating ROS/Ca2+ wave. Passage between cells may be mediated by either diffusion of ROS through the apoplast or Ca2+ through the plasmodesmata. Knock out mutant, tpc1, affects the Both the ABA-induced germination suppression and the stomatal response to extracellular Ca2+ are affected by functional SV channel activity. TPC1 contributes to cytosolic Ca2+ homeostasis indicated by ABA- and CO2-induced stomatal closure and ABA-, K+- and Ca2+-dependent root growth phenotypes were no different in tpc1 compared with wild-type plants. Additional methods are required to verify the protein localization and function in different plant species. Ca2+ signaling activity was found in AtTPC1, rice (OsTPC1) and wheat (TaTPC1).

**Annexins:**

Annexins are potential multifunctional proteins involved in regulating the trafficking of Ca2+ channel to a membrane, located and expressed in roots correspond well with the presence of a plasma membrane Ca2+ conductance that is involved in root cell elongation and is activated by hyperpolarization and extracellular hydroxyl radicals (OHc ; Demidchik et al., 2003; Foreman et al., 2003; Laohavisit et al., 2012). Knockout mutant was found to affect Ca2+ channel in epidermal and root hair apical plasma membrane of Arabidopsis thailiana (Atann1), with mutant root hairs found to be shorter than wild-type root hairs (Laohavisit et al., 2012). The role of an annexin is likely to be central to its contribution to Ca2+ signaling. How plant annexins can become extracellular remains to be determined (Laohavisit et al., 2011b; Clark et al., 2012), but their ability to increase [Ca2+]cyt by acting at the apoplast face of the plasma membrane has now been demonstrated (Laohavisit et al., 2009). The maize annexins found to form a Ca2+ conductance in PLB were also capable of transiently increasing the cytoplasmic calcium level of Arabidopsis protoplasts, but whether this was by directly forming a Ca2+ influx pathway or through the activation of other channels was not determined (Laohavisit et al., 2009). Purified maize annexins conduct Ca2+ across planar lipid bilayers (PLB), changing from being voltage independent to hyperpolarization activated when malondialdehyde is incorporated into the PLB to mimic lipid peroxidation (Laohavisit et al., 2009, 2010). Eight putative genes encoding for these cytoplasmic proteins and are found in Arabidopsis, and 25 and 11 genes were detected in wheat and barley, respectively (Xu et al., 2016). Two purified proteins ANN33 and ANN35 from the maize annexin family, , are permeable to both ions K+ and Ca2+ [135,136] and suggested that the annexin family could act as Ca2+ channels in calcium signaling processes.

**Mechanosensitive Calcium Permeable Channels ( MCA1 AND MCA2)**

Plants are constantly exposed to external mechanical stimuli, such as wind, compression, stretch, gravity, and touch, which sometimes indicate threat to plants, so mechanosensing and subsequent defense responses are particularly important for plants to survive, grow and develop under mechanically stressful conditions because of their sessile nature. Calcium permeable mechanosensitive channels being important component of mechano-sensing located in plasma membrane, endoplasmic reticulum, apoplast elicits an immediate calcium transient in the cytoplasm upon perception of mechanical stimuli. These calcium permeable channels were first identified in Arabidopsis (Nakagawa et al., 2007) and structurally these channels form homotetramer and have several motifs, such as an EF hand-like motif, coiled-coil motif, and plac8 (DUF614) motif as well as a few predicted putative transmembrane segments present in the cytosol. The EF hand-like motif can sense calcium transient in cytoplasm needed to regulate the activities of two plasma membrane proteins as putative Ca2+-permeable mechanosensitive channels, MCA1 (At4g35920) and MCA2 (At2g17780). Mechanosensitive proteins are also exhibited to mediate an increase in cytoplasmic calcium upon hypo-osmotic shock in Rice and Tobacco (Kurusu et al., 2012a: Kurusu et al., 2012b). Binding of calcium to EF-hand motifs, leading to deliberate production of reactive oxygen species (ROS) in the apoplast provide substrates for peroxidases to affect cell wall metabolism, cellular responses, including expression of mechanical stimulus-inducible genes (Takeda et al., (2008). Hypo-osmotic stress results in activation of calcium permeable mechanosensitive channel OsMCA1 to form calcium transient and production of ROS in cultured rice cells (Kurusu et al., 2012a: Kurusu et al., 2012b). Functions of MSLs include regulation of programmed cell death (MSL10; Veley et al., 2014), water and ion balance in pollen tubes (MSL8; Hamilton & Haswell, 2017).

**Calcium Sensors**

Upon perception of external biotic and abiotic stimuli, there is elevation of calcium concentration in cytoplasm, these alterations in the Ca2+concentration are sensed by Ca2+-binding proteins called calcium sensors or modulators. Calmodulin, calcium-dependent protein kinases, and calcineurin B-like proteins are a few classes of calcium binding sensory proteins found in plants that relay or decode encoded Ca2+ signals into particular cellular and physiological responses in order to survive environmental challenges (Aldon et al., 2018). These proteins have both protein kinase and calmodulin-like domains in a single polypeptide display various affinities for calcium ions and this property, combined with their sub-cellular location within the cell, will control their behaviour . Calcium binding to Ca2+ sensors will induce a conformational change that triggers either their association to downstream target proteins or a direct stimulation of the kinase activity when CPKs are considered (Harmon *et al.,* 2000). The diversity of Ca sensors and their downstream effects like altered protein phosphorylation and gene expression patterns contributes to a second layer of specificity, allowing the translation of various external cues into distinct biological responses (Hashimoto and Kudla, 2011).

**Calmodulin (CAM) and CAM Binding proteins (CAMBPS)**

Calmodulin (CaM) and calmodulin-like (CML) proteins are primary Ca2+ sensors involved in regulation of gene expression during plant immune responses. Calmodulin proteins sense the elevated Ca2+ levels and culminates Ca2+ signals into cellular responses through Ca2+-dependent regulation of downstream effectors. Calmodulins belong to a primary and prototypical class of calcium sensor in all eukaryotic cells. Sensor proteins possess 2 separate globular domans each having a pair of EF-hands motifs, a helix-loop-helix structure and each CaM binds to 4 Ca2+ ions. Generally, CaM has no catalytic activities of its own, but upon binding to Ca2+ via the EF-hand motif it changes its configuration leading to exposure of hydrophobic regions that form high affinity binding sites for downstream target proteins (Lecourieux *et al*., 2006). Hence, CaM functions by binding and regulating the activities of various downstream CaMBPs. Thus, CaMBPs provide another level of specificity for Ca2+ signaling since different CaMBPs trigger specific physiological responses (Cheval *et al.,* 2013). CaM also regulates other aspects of plant defense and upon pathogen attack there is induce rapid production of nitric oxide (NO) in plants, which serves as a modulator of disease resistance by triggering hypersensitive cell death and activating the expression of several defense genes (Hong et al., 2008). Another CaM-binding protein, CBP60g is believed to bind to and activate the expression of SA biosynthesis gene ICS1, providing a direct channel for Ca2+C signal to activate defense responseslike activation of stress response genes like chaperones, increase resistance of plant to SAR and stimulate production of antioxidants. s

**Calcium Dependent Protein Kinase (CDPK)**

Plants possess unique and ubiquitous calcium sensor CDPK involved in defense responses to abiotic and biotic stresses. Nicotiana tabaccum NtCDPK2 was first CDPK known to be involved in ETI triggered by the fungal elicitor Avr9 and another CDPK, AtCPK1 is able to phosphorylate phenylalanine ammonialyase (PAL) in vitro, an alternate pathway to produce SA. It contains a Ca2+ binding domain of 4 EF-hand motifs fused to the C-terminus of a Ser/Thr kinase domain with a junction of an autoinhibiotory domain (Harmon *et al.,* 2000) and the binding of Ca2+ to the EF-hand motif induces a configurational change leading to kinase activity. Therefore, changes in Ca2+ concentrations are translated into phosphorylation events and eventually to downstream physiological responses (Wernimont *et al.,* 2010). CDPKs are known to participate in hormone signaling, oxidative burst and gene expression network to regulate plant defense responses. Extracellular generation of ROS is a central component of the plants defense machinery. ROS act as direct toxicants to pathogens, catalyze early reinforcement of physical barriers and are involved in signaling later defense reactions, such as phytoalexin synthesis and defense gene activation, programmed cell death and protective reaction. Plays a critical role in the activation of defense responses. Increases in the levels of SA and its conjugates have been associated with the activation of resistance responses in a wide variety of plant species. These increases slightly precede or parallel the expression of PR genes in both the infected tissue as well as the uninfected tissues exhibiting SA

**Calcineurin B-Like Proteins (CBLS)**

CBLS are the third most significant plant specific small calcium binding proteins. These In order to decode Ca2+ signals, plant-specific Ca2+ sensor proteins have four EF-hand motifs as its Ca2+-binding domain and interact particularly with the CBL-interacting protein kinase (CIPK) family of Ser/Thr protein kinases (Kim et al., 2000). They interact with protein kinases as CBL-CIPK and interaction with CIPK alter the Ca2+binding properties of CBLs. Multiplicity of CBL/CIPK partnering provides for an impressive diversity of Ca2+responsive complexes. CBL2 and CBL4 proteins of Arabidopsis composed of two globular domains, each of which contains one EF hand pair, separated by a short linker region between the globular domains. During the evolution of plants the number of CBL and CIPK genes increased, suggesting that CBLs–CIPKs evolved concurrently with the adaptation and colonization process of plants on the land and with their increasing ability to cope with fluctuating environmental conditions. Crystal structure analysis of CBL2 from Arabidopsis revealed that the Ca2+ binding region of the EF hand is composed of 14 amino acids, instead of 12 amino acids as in canonical Ca2+-binding loops (Nagae et al, 2003). While CBL2 . The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis in Arabidopsis The calcium sensor CBL10 belongs to the family of calcineurin-B-like-proteins (CBLs) which specifically interact with a family of serine-threonine protein kinases designated as CBL-interacting protein kinases (CIPKs). CBL10 and CIPK24 constitute a novel Ca2+-regulated salt tolerance pathway that regulates the sequestration/compartmentalization of Na+ into vacuoles of green tissues. In response to diverse conditions like cold and salinity but not dehydration stress, it was discovered that both pea CBL and CIPK were coordinatedly increased. According to research by Torre et al. (2013), the calcineurin B-like protein 10 (Cbl10)/calcineurin B-like interacting protein kinase 6 (Cipk6) signaling module is implicated in ROS signaling during plant-pathogen interactions. Additionally, PTI (PAMP-triggered immunity) has been demonstrated to involve the CIPKs OsCIPK14 and OsCIPK15. In salt stress responses, CBL/CIPK function is achieved via complex formation of CIPK24 with either CBL1/CBL4 or CBL10, which results in a dual functioning kinase. At the plasma membrane of roots, CBL4-CIPK24 control Na+-extrusion through the Na+/H+ exchanger SOS1, whereas CBL10-CIPK24 complexes are localized at the vacuole in shoots where they may control Na+ sequestration into this organelle..

**Ca2+ Extrusion Systems:**

The cytosolic baseline [Ca2+]cyt increases several-fold as external Ca2+ activity increases by several orders of magnitude (Demidchik et al., 2002). The normal [Ca2+]cyt elevations for plant cell responses to phytohormones and environmental signals range from 0.5 to 10 lM. Even with these outside stimuli present, the basal [Ca2+]cyt concentration returns within a short period of time. Ca2+ extrusion and sequestration processes, which work against the electrochemical gradient, mediate this recovery, which calls for energy-intensive Ca2+ transporters. In plants, there are two different kinds of active Ca2+ transport systems: P-type Ca2+-ATPases that use the energy released during ATP hydrolysis (Bonza & De Michelis, 2011; Huda et al., 2013) and Ca2+/H+ exchangers of the CAX family (calcium/cation exchangers), which get their energy from the electrochemical gradient of protons across membranes facing the cytosol (Emery et al.

**Operation and regulation of plant Ca2+ATPases**:

Plant Ca2+ pumps belong to the P-type superfamily of ATPases and are thus energized by ATP hydrolysis by binding the c-phosphate of ATP to the aspartate residue within the DKTGT motif of the P-domain (Palmgren & Harper, 1999). This enzyme has two conformations known as the E1 and E2 states. The former has high affinity to Ca2+ and binds it at the cytosolic side of the membrane. After ATP hydrolysis and phosphorylation, the pump changes its conformation to the E2 state, with has a much lower affinity to Ca2+ and has an ion binding site located on the opposite (to cytosol) side (Kabala & Klobus, 2005). As a result, Ca2+ dissociates from the protein on the outer side of membrane. In general, the ATPasemediated mechanism is considered to be of a low capacity, with a high affinity for Ca2+ (Km = 0.01–2 lM; Huda et al., 2013a,b), and acts with a 1 : 1 Ca2+ to ATP stoichiometry (Lopreiato et al., 2014)

**Operation and regulation of CAXs**:

These proteins possess two cation-binding sites, termed a1- and a2-repeat regions, which are located within transmembrane helixes 2–3 and 7–8, respectively. It was shown that Ca2+ and H+ binding within these repeat regions are mutually exclusive (Nishizawa et al., 2013; Waight et al., 2013), suggesting the existence of a ‘one-H+ -in, oneCa2+-out’ exchange mechanism (Pittman & Hirschi, 2016). The protein structure is reset by H+ binding. Autoinhibition of CAX proteins is caused by the N-terminus physically interacting with a neighbouring N-terminal region (Manohar et al., 2011). Modulation of CAX activities could occur by phosphorylation, changes of pH and reaction to regulatory proteins such as the serine/threonine kinase SOS2 with the CAX N-terminal domain (Demidchik & Shabala, 2018)

**Conclusion:**

Following a pathogen challenge, changes in cytosolic Ca2+ contents in plant cells have been noticed and identified as an early event crucial for plant defensive responses. Identification and analysis of CaM-binding proteins, CDPKs and other calcium sensors revealed that Ca2+ signalling participates in diverse aspects of plant defense responses. Mechanosensitive channels have allowed the elucidation of their substantial roles not only in perception of mechanical stimuli, but also in programmed cell death, responses to salt stress, regulation of organelle shape and ROS sensing. Significant progress in our understanding of membrane calcium transport has been achieved over the last decade. Most Ca2+- permeable channels have been cloned and electrophysiologically tested in heterologous expression systems. Plasma membrane Ca2+-permeable channels interact with Ca2+- activated NADPH oxidase to form a self-amplifying system: a ROS-Ca2+ hub. This system could provide the transduction and amplification of the initial Ca2+ or ROS stimuli into a more sustainable response, with implications for cell growth, hormonal signalling and stress responses. Ca2+-extruding systems, the Ca2+-ATPases and Ca2+/H+ exchangers, operate in concert with Ca2+ -permeable channels to form a finely tuned mechanism for Ca2+ removal from the cytosol. The crystal structure of the Ca2+-ATPase autoinhibitory domain demonstrates a biphasic activity of this protein in Ca2+ extrusion, which increases with [Ca2+]cyt., thus explaining its action in signalling cascades. The role of other putative calcium exchangers, such as the calcium sodium like exchanger (Wang *et al*., 2012; Li *et al*., 2016), is also emerging but requires more detailed research. A large number of studies have been conducted using KO mutants and overexpressing lines of Ca2+-transporting systems, revealing critical roles of calcium transport systems in intracellular signalling, Ca2+ and Mg2+ nutrition, elongation growth, cytoskeleton regulation, biotic and abiotic stress responses, programmed cell death, gravity sensing, ROS, hormones, temperature changes, mechanical stimuli, control of stomatal closure and photosynthesis. All this makes the Ca2+-transporting machinery a highly attractive target for genetic improvement of plants for environmental fitness. However, the large number of Ca2+ transporting systems and the complexity of their regulation make the practical task of reprogramming stress resilience and control over plant development and productivity extremely challenging. Further progress in this direction may therefore only be achieved by comprehensive functional studies. This will reveal the role of a particular Ca2+ transporter encoding gene(s) in specific developmental and/or stress responses.

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