Review Article

Calcium Signalling: A key mediator in plant immune mechanism

ABSTRACT

Plants lack the adaptive immune system found in vertebrates and rely solely on innate defense composed of two interrelated layers: pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). The perception of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) activates PTI leading to early immune responses such as calcium (Ca^{2+}) influx and reactive oxygen species (ROS) burst. Conversely, pathogen-secreted effector proteins target host defense components to suppress PTI but their recognition by intracellular resistance (R) proteins triggers ETI and hypersensitive cell death. Calcium functions as a pivotal secondary messenger in both PTI and ETI, mediating the activation of downstream defense signalling networks. Plasma membrane channels such as cyclic nucleotide-gated channels (CNGCs) and glutamate receptor-like channels (GLRs) regulate Ca^{2+} influx while intracellular stores such as the vacuole and endoplasmic reticulum contribute additional Ca^{2+} release. Calcium sensors including calmodulins (CaMs), calcium-dependent protein kinases (CDPKs) and calcineurin B-like proteins (CBLs) decode these Ca^{2+} signals to modulate ROS bursts and hormone-mediated defenses involving salicylic acid (SA) and jasmonic acid (JA). This review synthesizes insights into calcium signalling in plant immunity emphasizing molecular players and signalling cross-talks.

Keywords: ROS, PTI, ETI, CNGC, CDPK, plant immunity, calcium signalling

1. INTRODUCTION

Plants are constantly exposed to numerous pathogens that invade their tissues, disrupt cellular functions and exploit host resources for their own advantage. Since plants do not have the adaptive immunity that animals do, they have an innate immune system to combat these threats. Pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) are the two interrelated layers that work together to coordinate a dynamic and multilayered defense response ~~are the two main components of plant immunity~~ (Jones & Dangl, 2006; Ngou *et al*., 2021).

When microbial signatures known as pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) are recognized such as bacterial flagellin or fungal chitin, PTI, the first line of defense is activated. Pattern recognition receptors (PRRs), which are found on the plasma membrane of plant cells are responsible for identifying these patterns (Zipfel, 2014; Couto & Zipfel, 2016). The PAMP–PRR interaction starts a series of cellular reactions such as the release of reactive oxygen species (ROS), the activation of mitogen-activated protein kinase (MAPK) cascades, the rapid influx of calcium ions (Ca^{2+}) and transcriptional reprogramming (Seybold *et al*., 2014; Boller & Felix, 2009; Kadota *et al*., 2014). In order to restrict pathogen entry and growth, these reactions strengthen biochemical and structural barriers (Wan *et al*., 2021).

Virulent pathogens can suppress PTI by secreting effector proteins directly into the cytoplasm of the host. Plants respond by deploying ETI, a second layer of immunity which is mediated by resistance (R) proteins, primarily nucleotide-binding leucine-rich repeat (NLR) receptors. These proteins initiate a strong immune response when they identify the existence or activity of effectors (Cui *et al*., 2015; Ngou *et al*., 2022). The hypersensitive response (HR), a type of localized programmed cell death that inhibits pathogen spread and triggers systemic acquired resistance frequently coexists with ETI (Nguyen *et al*., 2021; Coll *et al*., 2011).

Calcium signalling plays a key role in both PTI and ETI. A crucial secondary messenger calcium influx coordinates downstream reactions like hormone signalling, defense gene activation and ROS burst (Dodd *et al*., 2010; Tian *et al*., 2020). The spatiotemporal patterns of cytosolic calcium elevations during immune responses are controlled by particular calcium channels and transporters such as glutamate receptor-like channels (GLRs), cyclic nucleotide-gated channels (CNGCs) and tonoplast-localised calcium pumps (Wang *et al*., 2017; Moeder *et al*., 2011). Calcium-binding proteins like calcineurin B-like proteins (CBLs), calcium-dependent protein kinases (CDPKs) and calmodulins (CaMs) decode these calcium signatures and convert the signal into specific immune outputs (Luan *et al*., 2002; Boudsocq *et al*., 2010).

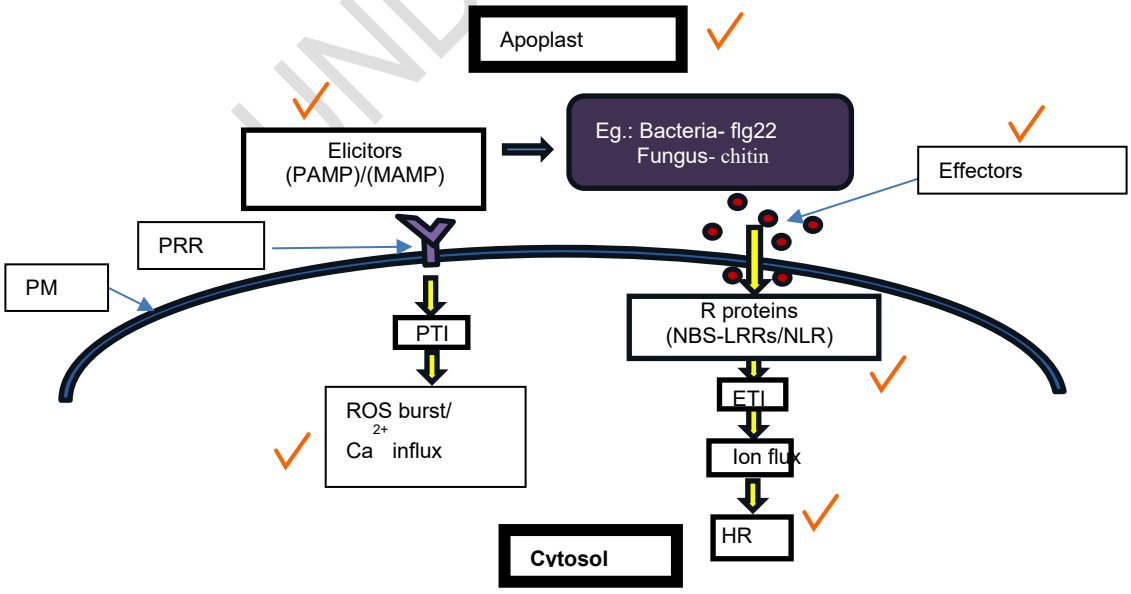

Fig. 1. Schematic representation of PTI and ETI signalling pathways in plant innate immunity

2. ROLE OF CALCIUM IN PLANT IMMUNITY

As a universal secondary messenger in signalling pathways that coordinate defense responses, calcium (Ca^{2+}) plays a crucial role in plant immunity. A rapid influx of Ca^{2+} occurs when a pathogen is recognised, either by PRRs in PTI or by NLRs in ETI. Both extracellular and intracellular reservoirs including the vacuole, apoplast and endoplasmic reticulum serve as sources of this Ca^{2+} influx (Toyota *et al*., 2018; Thor *et al*., 2020; Yang *et al*., 2019). Reference missing

A network of calcium-binding proteins such as CBLs, CDPKs and CaMs sense and interpret the transient increase in cytosolic Ca^{2+} concentration (Hashimoto and Kudla, 2011; Kudla *et al*., 2018). These calcium sensors function as molecular decoders that transduce Ca^{2+} signatures into immune signals leading to activation of ROS production, transcription of defense-related genes and strengthening of the cell wall (Dubiella *et al*., 2013; Aldon *et al*., 2018).

Calcium-dependent phosphorylation mediated by CDPKs directly activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidases such as respiratory burst oxidase homolog D (RBOHD) promoting oxidative bursts critical for pathogen restriction (Kadota *et al*., 2014). Meanwhile, CaM- and CBL–CIPK-mediated cascades regulate ion homeostasis and fine-tune immune gene expression to ensure balanced defense signalling (Meena and Vadassery, 2015; Ma *et al*., 2020). Cite Fig 3

Tight regulation of the temporal and spatial dynamics of calcium signalling ensures that immune responses remain localized and proportional to the stimulus. Through these finely tuned mechanisms, calcium serves as a vital signal transducer that allows plants to mount rapid, specific and effective defenses against a wide range of pathogens (Bhar *et al*., 2023; Stael *et al*., 2012).

3. INITIATION OF CALCIUM SIGNALS

One of the earliest immune reactions in plants is the initiation of calcium (Ca^{2+}) signalling upon pathogen recognition. This process begins with the activation of specific calcium-permeable channels in the plasma membrane, primarily GLRs and CNGCs which facilitate rapid Ca^{2+} entry into the cytoplasm from the apoplast or intracellular stores such as the vacuole (K Jha *et al*., 2016; Saijo and Loo, 2020).

CNGCs play a pivotal role in mediating Ca^{2+} influx during plant immune responses. These channels are activated when cyclic nucleotides, including cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) bind to their cyclic nucleotide-binding domain (CNBD) inducing conformational changes that open the channel pore (Moeder *et al*., 2011; Duszyn *et al*., 2019). CNGCs respond to diverse stimuli including pathogen perception, abiotic stress and hormonal signals such as abscisic acid (ABA) and salicylic acid (SA) linking multiple signalling pathways to Ca^{2+} homeostasis (Jha *et al*., 2021).

The resulting Ca^{2+} influx activates calcium-binding proteins such as CaM, CBLs and CDPKs which subsequently regulate downstream immune responses including transcriptional reprogramming, reactive oxygen species (ROS) production, cell wall fortification and stomatal closure (Tuteja and Mahajan, 2007).

GLRs are another important class of calcium channels which are activated by extracellular amino acids, peptides or other ligands and share structural similarity with animal ionotropic glutamate receptors (Vincill *et al*., 2013). Once activated, GLRs contribute to amplifying the Ca^{2+} signal by promoting sustained influx into the cytosol thereby reinforcing the early immune signal (Yu *et al*., 2022; Grenzi *et al*., 2022).

The coordinated activity of CNGCs and GLRs leads to a sharp and transient increase in cytosolic Ca^{2+} concentration. This Ca^{2+} signature is subsequently decoded by sensor proteins triggering cascades that integrate with ROS bursts, MAPK activation and defense gene expression to establish robust immune responses (Xiao *et al*., 2023). The timing, amplitude and localization of these calcium signals are critical in determining the specificity and strength of the plant's defense strategy against invading pathogens (Thor *et al*., 2020).

Fig 2

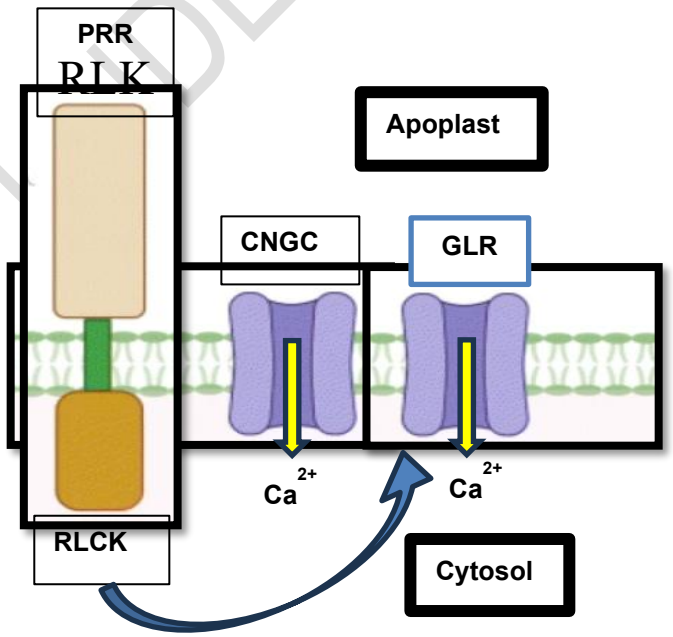

Fig. 2. PRR–RLCK signalling mediates Ca^{2+} influx through CNGC and GLR channels

4. CELL ORGANELLES AND STRUCTURES PARTICIPATING IN CYTOSOLIC CALCIUM INFLUX

Apart from plasma membrane channels, intracellular organelles such as the endoplasmic reticulum (ER) and vacuole play indispensable roles in regulating cytosolic calcium (Ca^{2+}) levels during immune responses. The endoplasmic reticulum serves as one of the major intracellular Ca^{2+} reservoirs maintaining a steep concentration gradient relative to the cytosol. Upon pathogen recognition, immune signalling cascades frequently activate phospholipase C (PLC) which catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) (Bootman and Bultynck, 2020). The IP_3 molecule binds to its receptors (IP_3Rs) located on the ER membrane, triggering the release of Ca^{2+} into the cytosol (Xu *et al*., 2022). Ryanodine receptor-like (RYR) channels further enhance this release promoting rapid and sustained cytosolic Ca^{2+} elevations that are vital for activating downstream immune signalling pathways including ROS production, MAPK activation and defense gene transcription (Sanyal *et al*., 2019).

Similarly, the vacuole which is the largest organelle in plant cells acts as an additional dynamic Ca^{2+} store that contributes significantly to intracellular calcium homeostasis. The two-pore channel 1 (TPC1) located on the tonoplast mediates vacuolar Ca^{2+} efflux in response to electrical or chemical cues associated with biotic stress (Peiter *et al*., 2005). During immune activation, TPC1 releases Ca^{2+} into the cytoplasm reinforcing the ER-derived calcium signal and sustaining cytosolic Ca^{2+} elevations necessary for long-term immune signalling (Kintzer and Stroud, 2018). This vacuolar contribution not only amplifies defense-associated calcium transients but also plays a vital role in maintaining cellular ion equilibrium during prolonged stress (Pantoja, 2021).

The coordinated calcium release from both ER and vacuole ensures that the temporal and spatial patterns of Ca^{2+} signalling are tightly controlled. This integration fine-tunes immune responses, enabling plants to balance signal amplification with homeostatic regulation, thereby preventing cytotoxic calcium overload while ensuring robust defense activation (Stael *et al*., 2012; Choi *et al*., 2014).

5. DETECTION OF CALCIUM INFLUX

The entry of calcium ions (Ca^{2+}) into the cytosol in response to pathogen attack acts as a critical signal that initiates a multitude of defense mechanisms in plants. A diverse network of calcium-binding proteins senses this transient rise in cytosolic Ca^{2+} concentration, each performing distinct yet coordinated roles in decoding the signal into specific molecular and physiological outputs (Kudla *et al*., 2018; Dodd *et al*., 2010).

5.1 *Calcium-Dependent Protein Kinases (CDPKs)*

Calcium-dependent protein kinases (CDPKs) also referred to as CPKs represent a unique class of serine/threonine kinases that directly couple calcium sensing with downstream phosphorylation events. CDPKs contain both a kinase domain and a calmodulin-like regulatory domain with EF-hand motifs that bind Ca^{2+} ions inducing conformational changes that activate kinase activity (Dubiella *et al*., 2013). Once activated, CDPKs phosphorylate diverse substrates including metabolic enzymes, transcription factors, NADPH oxidases and other signalling intermediates (Boudsocq and Sheen, 2013). These phosphorylation cascades modulate immune signalling, hormone regulation and defense gene expression. For example, *Arabidopsis* CPK5 and CPK6 have been shown to regulate the activation of WRKY transcription factors and ROS bursts in pathogen defense (Romeis and Herde, 2014; Zhou *et al*., 2020). CDPKs thus serve as key hubs integrating Ca^{2+} signals into transcriptional and metabolic responses during immunity (Yip Delormel and Boudsocq, 2019).

5.2 *Calmodulins (CaMs)*

Calmodulins (CaMs) are highly conserved calcium sensors that interpret transient Ca^{2+} oscillations into functional responses. Upon Ca^{2+} binding, CaMs undergo structural rearrangements that enable interaction with a broad range of target proteins including kinases, phosphatases and metabolic enzymes (Cheval *et al*., 2013; Zhang *et al*., 2014). Through these interactions, CaMs participate in stomatal regulation, systemic acquired resistance and hormone signalling pathways such as salicylic acid and jasmonate signalling (Ranty *et al*., 2006; Zeng *et al*., 2023). Additionally, CaMs activate calcium/calmodulin-dependent protein kinases (CaMKs) further amplifying Ca^{2}-dependent transcriptional responses (Perochon *et al*., 2011). In plant immunity, specific CaM isoforms modulate the activity of defense-related transcription factors and nitric oxide synthases, fine-tuning immune activation (Poovaiah *et al*., 2013).

5.3 *Calcineurin B-Like Proteins (CBLs)*

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Calcineurin B-like proteins (CBLs) function as specialized Ca^{2+} sensors that transmit calcium signals through their interaction with CBL-interacting protein kinases (CIPKs). Like CaMs and CDPKs, CBLs contain EF-hand motifs for calcium binding but they specifically localize to cellular membranes where they recruit CIPKs to regulate ion transport and stress signalling (Batistič *et al*., 2009; Chen *et al*., 2024). The CBL–CIPK network is essential for maintaining ion homeostasis, controlling K^+ and Na^+ transporters and coordinating responses to both biotic and abiotic stresses (Steinhorst and Kudla, 2013; Ketehouli *et al*., 2019). In plant immunity, CBL–CIPK modules modulate membrane potential, ROS production and defense gene regulation in response to calcium fluctuations (Tang *et al*., 2020). This finely tuned system enables plants to translate intracellular calcium signatures into adaptive defensive outcomes.

6. CALCIUM SENSOR-MEDIATED ROS BURST

An essential regulatory axis in the oxidative burst, a defining feature of plant immune responses, is formed by the interaction of calcium sensors, respiratory burst oxidase homologs (RBOHs) and calcium-dependent protein kinases (CDPKs).

Plants show a quick influx of calcium ions (Ca^{2+}) into the cytosol when they recognize a pathogen or are under environmental stress. By acting as a secondary messenger, this increase in cytosolic calcium starts subsequent defense signalling pathways (Dubiella *et al*., 2013; Kadota *et al*., 2015). As serine/threonine kinases and calcium sensors, CDPKs pick up on these variations through EF-hand motifs that bind Ca^{2+} causing conformational changes that activate their kinase domains.

The NADPH oxidase family also known as RBOHs (Respiratory Burst Oxidase Homologs) is a major target of activated CDPKs. The production of reactive oxygen species (ROS), specifically the superoxide anion ($O_2^{\bullet -}$) is catalysed by these membrane-bound enzymes and subsequently dismutated into hydrogen peroxide (H_2O_2) (Marcec and Tanaka, 2021; Zhang *et al*., 2014). Certain serine residues on RBOH proteins are phosphorylated by CDPKs, activating them and starting the oxidative burst (Ogasawara *et al*., 2008; Boudsocq *et al*., ~~2010~~ 2013).

This pathway produces ROS, which have several interrelated functions in plant immunity:

Type text here

1. Direct antimicrobial action that breaks down the proteins and membranes of pathogens.

2. Cell wall fortification through the encouragement of structural component oxidative cross-linking.

3. Defense signalling, since ROS act as auxiliary messengers to enhance immune reactions (Torres, 2010).

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4. To contain the infection, the hypersensitive response (HR) is induced which results in localized programmed cell death (Gilroy *et al*., 2016).

Achieving a balance between preventing collateral damage to host tissues and providing effective pathogen defense requires this tightly controlled ROS burst. CDPKs are essential transducers that connect early calcium signalling to oxidative responses by activating RBOHs in a Ca^{2+}-dependent manner (Kadota *et al*., 2015). In conclusion, CDPKs' capacity to sense calcium allows pathogen-induced calcium signals to be quickly translated into targeted phosphorylation of NADPH oxidases, coordinating a controlled oxidative burst that is crucial for plant defense (Marcec and Tanaka, 2021; Kadota *et al*., 2015).

Cite Fig 3

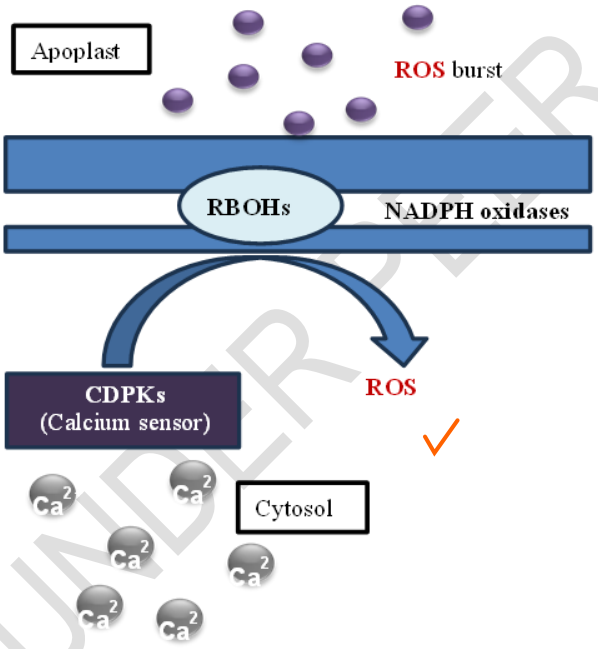

Fig. 3. Ca^{2+}-dependent activation of RBOH-driven ROS production in the apoplast

7. MECHANISM OF CALCIUM SIGNALLING PATHWAY

A key component of plant reactions to biotic and abiotic stimuli is calcium (Ca^{2+}) signalling. Calcium-permeable channels on the plasma membrane, vacuole and endoplasmic reticulum (ER) are activated when pattern recognition receptors (PRRs) on the plasma membrane recognize external signals such as pathogen-associated molecular patterns (PAMPs). Ca^{2+} enters the cytosol quickly and temporarily as a result of this activation (Reddy *et al*., 2011; Stael *et al*., 2012).

Calcium sensor proteins such as calmodulin (CaM) and calcium-dependent protein kinases (CDPKs) sense the subsequent increase in cytosolic Ca^{2+} concentration. By starting downstream reactions, particularly the activation of NADPH oxidases (RBOHs) which catalyze the generation of reactive oxygen species (ROS), these sensors transduce the signal (Dubiella *et al*., 2013; Kadota *et al*., 2015). These ROS enhance the defense response which includes hypersensitive cell death, gene expression modulation and cell wall reinforcement by acting as secondary messengers and antimicrobial agents (Torres, 2010; Gilroy *et al*., 2016).

Crucially, calcium signalling interacts with two important hormone pathways that are essential for pathogen defense such as salicylic acid (SA) and jasmonic acid (JA). Calcium-dependent protein kinases and calmodulins regulate several enzymes and transcription factors involved in SA and JA biosynthesis and signalling (Du *et al*., 2009; Seybold *et al*., 2014). CaM-binding transcription activators (CAMTAs), for instance can either positively or negatively regulate SA-dependent gene expression influencing the activation of systemic acquired resistance (SAR) (Kim *et al*., 2017). Similarly, CDPK-mediated phosphorylation modulates JA-responsive gene expression by affecting MYC transcription factors and JAZ repressors (Asano *et al*., 2012; Khan *et al*., 2023).

This intricate cross-talk ensures that calcium signalling acts as a key integrator between early pathogen perception and hormonal defense responses. Through these dynamic interactions, Ca^{2+} functions not only as a rapid secondary messenger but also as a regulatory hub coordinating local and systemic immune responses to pathogen attack (Bigeard *et al*., 2015; Poovaiah *et al*., 2013).

7.1 *Calcium and the SA Pathway*

Genes involved in salicylic acid (SA) biosynthesis are expressed more frequently when Ca^{2+}-activated kinases such as calcium-dependent protein kinases (CDPKs) and mitogen-activated protein kinases (MAPKs) are present. The isochorismate pathway in chloroplasts, catalyzed mainly by ISOCHORISMATE SYNTHASE 1 (ICS1) is the principal route through which SA is synthesized from chorismate (Dempsey *et al*., 2011). CDPKs and MAPKs through phosphorylation of

transcription factors like WRKY and CAMTA family members, enhance ICS1 expression thereby promoting SA accumulation (Du *et al*., 2009; Seybold *et al*., 2014).

When SA accumulates, it activates NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1), a central regulator of SA-mediated transcription leading to the induction of pathogenesis-related (PR) genes (Wu *et al*., 2012). This cascade ultimately contributes to systemic acquired resistance (SAR), a long-lasting immune state that provides broad-spectrum protection against subsequent pathogen attacks (Cui *et al*., 2015; Kim *et al*., 2017). Calcium's role in fine-tuning SA production and NPR1 activation highlights its importance as a key secondary messenger linking early recognition events to transcriptional immunity responses (Roychowdhury *et al*., 2024).

7.2 *Calcium and the JA Pathway*

Conversely, in the jasmonic acid (JA) pathway transcription factors such as MYC2 are modulated by CDPK-mediated Ca^{2+} signalling, influencing the expression of genes required for JA biosynthesis and response (Asano *et al*., 2012). JA biosynthesis follows the octadecanoid pathway, beginning in the chloroplast where α-linolenic acid is converted into 12-oxo-phytodienoic acid (OPDA) which is subsequently transformed into JA within the peroxisomes (Wasternack and Hause, 2013).

Calcium-dependent phosphorylation events can regulate enzymes in this pathway and modulate JA signalling components, including JASMONATE ZIM-DOMAIN (JAZ) repressors and MYC transcription factors (Asano *et al*., 2012). Consequently, JA-mediated defenses are activated, promoting resistance against necrotrophic pathogens and herbivorous insects. CDPKs also interact with other calcium-binding proteins such as CaM and calcineurin CBLs to fine-tune JA-dependent gene expression and cross-communicate with SA signalling (Poovaiah *et al*., 2013; Bigeard *et al*., 2015).

7.3 *Integration and Homeostasis*

Plants modulate their immune responses according to the invading pathogen type by integrating SA and JA signalling through calcium-dependent networks. SA typically confers defense against biotrophic pathogens while JA is associated with responses to necrotrophs and insect herbivores (Pieterse *et al*., 2012; Bari and Jones, 2009). This antagonistic yet complementary relationship is coordinated by Ca^{2+}-mediated transcriptional and post-translational regulation mechanisms.

Organelles such as the vacuole, endoplasmic reticulum (ER), chloroplasts and mitochondria act as dynamic reservoirs and modulators of Ca^{2+} and reactive oxygen species (ROS), influencing calcium signalling homeostasis (Stael *et al*., 2012). After

defense activation, calcium ATPases (such as ACA and ECA families) and Ca^{2+}/H^{+} antiporters actively restore basal cytosolic Ca^{2+} levels, maintaining spatiotemporal precision in signal transduction (Berridge *et al.*, 2003). This regulation ensures effective defense activation without triggering excessive or prolonged calcium stress that could compromise cellular integrity.

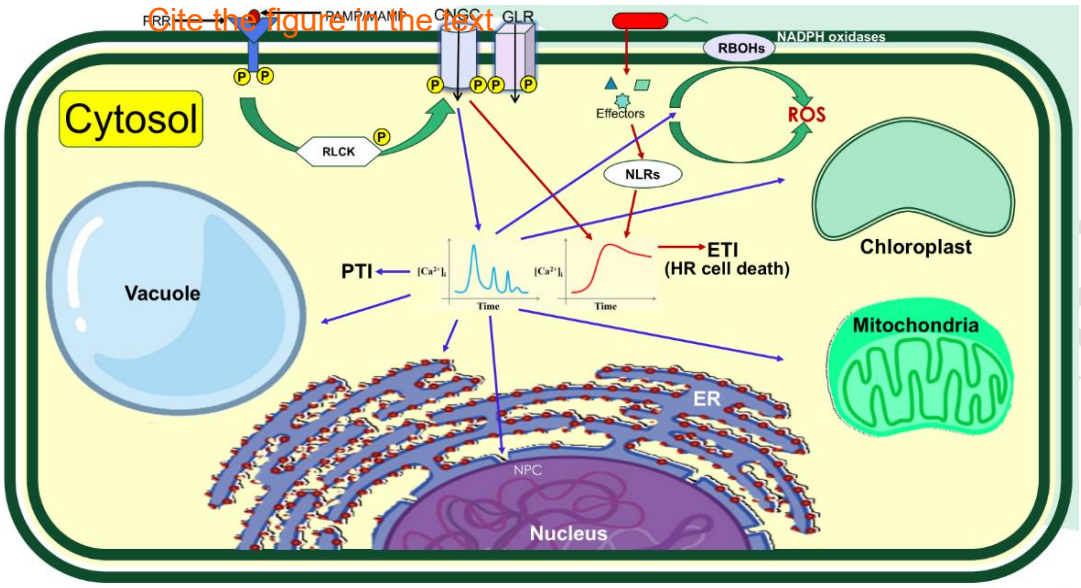

Fig. 4. Schematic representation of intracellular Ca^{2+} flux and ROS signalling integrating PTI and ETI responses in plant cells. PRR- and NLR-mediated pathways activate ion channels, NADPH oxidases and organelle communication, leading to immune signalling and hypersensitive cell death.

8. CALCIUM SIGNALLING-INDUCED DEFENSE RESPONSES IN PLANTS

In plant defense, calcium ions (Ca^{2+}) serve as ubiquitous second messengers, converting pathogenic and environmental stimuli into a variety of cellular reactions. Calcium channels on the plasma membrane, tonoplast (vacuolar membrane) and endoplasmic reticulum (ER) open in response to pathogen perception or abiotic stress causing a quick and temporary rise in cytosolic Ca^{2+} concentration. Both locally at the attack site and systemically throughout the plant, this increase in intracellular Ca^{2+} serves as the main signal that triggers subsequent defense reactions.

8.1 *Activation of Calcium Sensors and Signal Transduction*

Calcium-binding proteins such as calcineurin B-like proteins (CBLs), calcium-dependent protein kinases (CDPKs) and calmodulins (CaMs) act as sensors that detect the transient increase in cytosolic Ca^{2+} levels following pathogen perception. These sensors initiate phosphorylation cascades involving CDPKs and mitogen-activated protein kinases (MAPKs) which in turn regulate the activity of key defense-related transcription factors such as WRKYs, NPR1 and MYC2 (Boudsocq and Sheen, 2013). Once activated, these transcription factors translocate to the nucleus and modulate the expression of defense-associated genes including those involved in phytoalexin biosynthesis, cell wall reinforcement and pathogenesis-related (PR) protein production (Bigeard *et al*., 2015).

8.2 *Transcriptional Reprogramming and Cell Wall Fortification*

Calcium signalling orchestrates extensive transcriptional reprogramming that strengthens the plant's structural and biochemical defenses. Activation of genes encoding callose synthase and lignin biosynthesis enzymes results in the deposition of callose (β-1,3-glucan) and lignin at infection sites (Zhang *et al*., 2014). These polymers fortify the cell wall, forming a mechanical barrier against pathogen invasion. Simultaneously, calcium-mediated activation of transcription factors enhances the production of antimicrobial peptides such as defensins which provide direct inhibition of pathogen proliferation (Checker *et al*., 2018).

8.3 *Calcium-ROS Crosstalk and Hypersensitive Response*

Calcium signalling is closely associated with the generation of ROS which play a dual role in direct pathogen restriction and signal amplification. Activation of NADPH oxidases, primarily RBOHs triggers a rapid oxidative burst at infection sites (Marino *et al*., 2012). This ROS accumulation induces the hypersensitive response (HR), a form of programmed cell death that confines pathogens by limiting nutrient access (Levine *et al*., 1994). Furthermore, ROS act as mobile signals that propagate to neighbouring cells, priming them for enhanced immunity. ROS also contribute to cross-linking of cell wall proteins, thereby reinforcing cellular integrity (Torres, 2010).

8.4 *Hormonal Crosstalk: Salicylic Acid and Jasmonic Acid Pathways*

Salicylic acid (SA) and jasmonic acid (JA), two hormones involved in plant defense, are crucially integrated by calcium signalling.

8.4.1 *Salicylic Acid (SA) Pathway*

In biotrophic pathogen defense, calcium signalling stimulates the isochorismate pathway of SA biosynthesis through Ca^{2+}-dependent activation of WRKY and CAMTA transcription factors (Du *et al*., 2009). Accumulated SA activates NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1), leading to the induction of PR genes and the establishment of systemic acquired resistance (SAR), a long-lasting and broad-spectrum immune state (Wu *et al*., 2012; Kim *et al*., 2017).

8.4.2 *Jasmonic Acid (JA) Pathway*

In contrast, JA regulates defenses against necrotrophic pathogens and herbivorous insects. It is synthesised via the octadecanoid pathway from α-linolenic acid to 12-oxo-phytodienoic acid (OPDA) and finally to JA in peroxisomes (Wasternack and Hause, 2013). Calcium-dependent phosphorylation influences key enzymes in this pathway and modulates the stability of JAZ repressors. Degradation of JAZ proteins releases transcription factors such as MYC2 which activate genes encoding defense enzymes, proteinase inhibitors and volatile organic compounds (VOCs) involved in indirect defense (Kim *et al*., 2025).

8.5 *Stomatal Closure and Apoplastic Defense*

Ca^{2+} signalling plays a central role in pre-invasion defense mechanisms such as stomatal closure. Pathogen-associated molecular patterns (PAMPs), including flagellin (flg22), trigger Ca^{2+} influx in guard cells activating S-type anion channels and plasma membrane H^+-ATPases that mediate stomatal closure, thereby preventing pathogen entry (Melotto *et al*., 2006; Thor *et al*., 2020). Calcium signalling also regulates the synthesis of pectins, extensins and other structural proteins, enhancing the elasticity and resilience of cell walls against enzymatic degradation by pathogens (Oelmüller *et al*., 2023).

8.6 *Systemic Signalling and Acquired Immunity*

Beyond local defense responses, Ca^{2+} serves as a long-distance signalling molecule that propagates immune activation throughout the plant. Systemic transmission of calcium waves often accompanied by ROS and defense hormones

occurs through the phloem resulting in systemic priming of distal tissues (Toyota *et al*., 2018). This signalling contributes to the establishment of systemic acquired resistance (SAR) providing durable, whole-plant protection against future infections (Cui *et al*., 2015).

8.7 *Signal Termination and Homeostasis*

To prevent calcium toxicity and sustain responsiveness, plants employ active homeostatic mechanisms following defense activation. Ca^{2+}-ATPases and Ca^{2+}/H^{+} antiporters located on the plasma membrane, endoplasmic reticulum and vacuole rapidly restore basal Ca^{2+} levels by re-sequestering or exporting ions (Brini *et al*., 2012). These processes ensure the transient nature of the Ca^{2+} signal and maintain cellular equilibrium, allowing the system to remain primed for subsequent stress stimuli (Berridge *et al*., 2003).

9. CALCIUM CONCENTRATIONS IN CELLULAR COMPARTMENTS DURING PLANT IMMUNE RESPONSES

In plant cells, calcium (Ca^{2+}) homeostasis is tightly regulated, maintaining distinct concentration gradients across cellular compartments under both resting and stress conditions. Upon pathogen recognition, a coordinated release of Ca^{2+} from internal stores and an influx from the apoplast lead to a rapid cytosolic calcium elevation forming the core of calcium-mediated immune signalling cascades (Dodd *et al*., 2010; McAinsh and Pittman, 2009). The compartment-specific dynamics of Ca^{2+} are outlined below:

9.1 *Cytosol*

Concentration at rest is approximately 100–200 nM. During immune response: rapidly rises to 1–10 µM typically peaking within minutes of pathogen perception. The cytosol serves as the central hub for Ca^{2+} signal integration. The transient spikes in cytosolic Ca^{2+} are detected by calcium-binding proteins such as CaMs and CDPKs which initiate downstream defense responses including ROS generation, transcriptional reprogramming and hormone signalling (Cheval *et al*., 2013; Boudsocq and Sheen, 2013).

9.2 *Apoplast (Extracellular Space)*

Concentration at rest is approximately 1–10 mM. During immune response it acts as the primary source of Ca^{2+} influx into the cytosol through plasma membrane-localised Ca^{2+} channels such as CNGCs and GLRs. The steep concentration gradient between the apoplast and cytosol facilitates the rapid entry of Ca^{2+} upon PAMP-triggered channel activation constituting one of the earliest steps in defense signalling (Checker *et al*., 2018; Thor *et al*., 2020).

9.3 *Endoplasmic Reticulum (ER)*

Resting concentration is approximately 50–500 µM. During immune response it releases Ca^{2+} into the cytosol via inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptor-like channels (RYRs). The ER acts as a major internal Ca^{2+} reservoir that shapes the amplitude and duration of cytosolic Ca^{2+} signals during stress contributing to the sustained activation of immune pathways (Carreras-Sureda *et al*., 2018).

9.4. *Vacuole*

Concentration at rest is approximately 0.1–1 mM. During immune response Ca^{2+} is released through tonoplast-localised channels including Ca^{2+}/H^+ antiporters and two-pore channels (TPC1). The vacuole not only serves as a dynamic Ca^{2+} reservoir but also buffers prolonged or systemic Ca^{2+} signals, maintaining ionic balance and supporting long-term defense responses (Peiter *et al*., 2005; Kiep *et al*., 2015).

9.5. *Chloroplasts*

Concentration at rest is approximately 100–500 nM. During immune response transient Ca^{2+} fluctuations occur in response to biotic and abiotic stimuli, though detailed mechanisms remain less understood. Emerging evidence suggests that chloroplast Ca^{2+} signalling regulates retrograde communication with the nucleus modulates ROS production and contributes to the activation of salicylic acid (SA)-dependent defense gene expression (Stael *et al*., 2012).

9.6. *Mitochondria*

Concentration at rest is approximately 100–500 nM. During immune response mitochondria transiently accumulate Ca^{2+} acting as buffers and supporting ATP generation through Ca^{2+}-dependent metabolic enzymes. Mitochondrial Ca^{2+} fluxes are linked with ROS production and programmed cell death (PCD), coordinating energy supply and defense activation during pathogen challenge (Dey *et al*., 2020).

9.7 *Restoring Calcium Homeostasis*

Following the transient rise in cytosolic Ca^{2+}, specialized transport systems re-establish resting conditions. Ca^{2+}-ATPases and Ca^{2+}/H^{+} exchangers located on the plasma membrane, ER and tonoplast actively pump excess Ca^{2+} back into internal compartments or out of the cell (Brini *et al*., 2012). This restoration ensures that calcium signals are brief yet specific, maintaining cellular viability and preparing the plant for subsequent stimuli (Berridge *et al*., 2003).

(Fig.5)

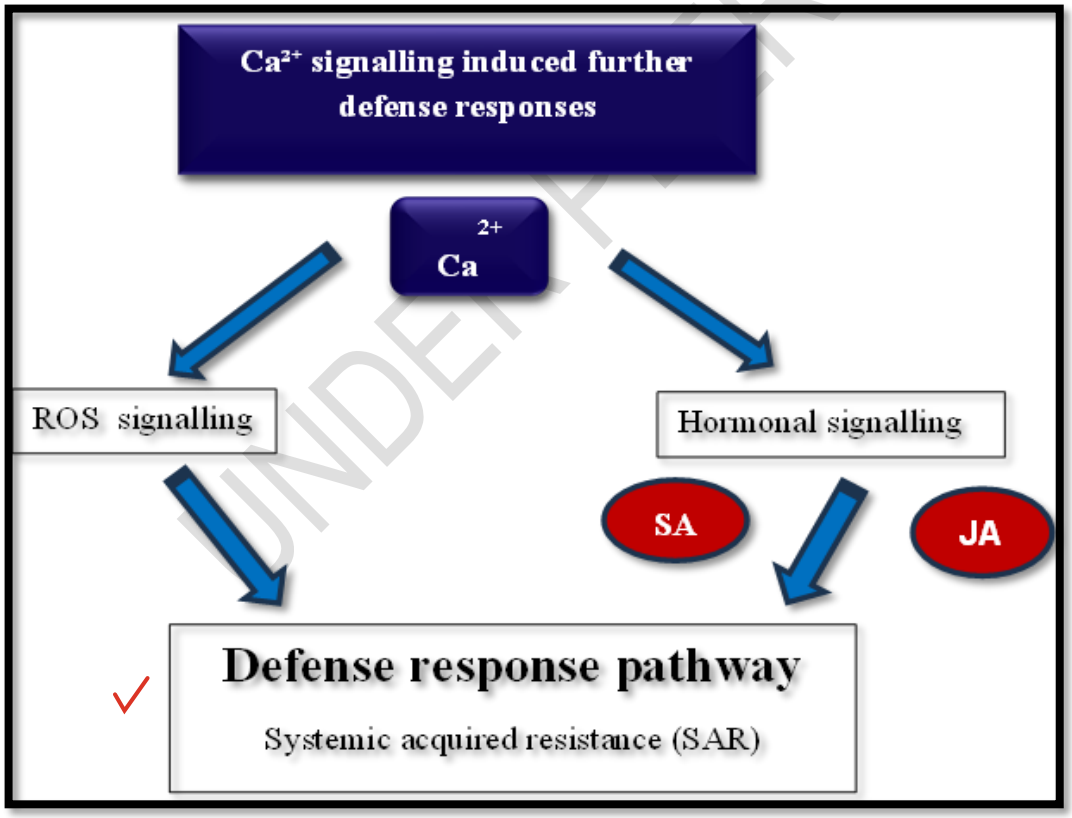

Fig. 5. Calcium signalling cascade leading to activation of SA, JA and related defense mechanisms

10. CONCLUSION

Calcium signalling is unequivocally a central hub in the plant immune network acting as a critical secondary messenger that translates the perception of pathogens into rapid and effective defense responses (Poovaiah *et al*., 1993; Trewavas, 1998). Foundational studies established Ca^{2+} as a universal second messenger in plants and subsequent work formalised the idea that information is contained in the *signature* of the Ca^{2+} signal — its amplitude, frequency, duration and subcellular localization (Dodd *et al*., 2010; McAinsh and Pittman, 2009). Distinct Ca^{2+} patterns have well-documented functional outcomes for example, repetitive oscillatory spiking in symbiotic signalling contrasts with the single sustained spike associated with certain hypersensitive responses (Oldroyd and Downie, 2006; Grant *et al*., 2000).

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