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REVIEW

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Calmodulin and calmodulin-like protein-mediated plant responses to biotic stresses

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Abstract

Plants have evolved a set of finely regulated mechanisms to respond to various biotic stresses. Transient changes in intracellular calcium (Ca^{2+}) concentration have been well documented to act as cellular signals in coupling environmental stimuli to appropriate physiological responses with astonishing accuracy and specificity in plants. Calmodulins (CaMs) and calmodulin-like proteins (CMLs) are extensively characterized as important classes of Ca^{2+} sensors. The spatial-temporal coordination between Ca^{2+} transients, CaMs/CMLs and their target proteins is critical for plant responses to environmental stresses. Ca^{2+}-loaded CaMs/CMLs interact with and regulate a broad spectrum of target proteins, such as ion transporters (including channels, pumps, and antiporters), transcription factors, protein kinases, protein phosphatases, metabolic enzymes and proteins with unknown biological functions. This review focuses on mechanisms underlying how CaMs/CMLs are involved in the regulation of plant responses to diverse biotic stresses including pathogen infections and herbivore attacks. Recent discoveries of crucial functions of CaMs/CMLs and their target proteins in biotic stress resistance revealed through physiological, molecular, biochemical, and genetic analyses have been described, and intriguing insights into the CaM/CML-mediated regulatory network are proposed. Perspectives for future directions in understanding CaM/CML-mediated signalling pathways in plant responses to biotic stresses are discussed. The application of accumulated knowledge of CaM/CML-mediated signalling in biotic stress responses into crop cultivation would improve crop resistance to various biotic stresses and safeguard our food production in the future.

KEYWORDS

calcium signal, calmodulin-binding protein, defense response, herbivore, pathogen

1 | INTRODUCTION

As sessile organisms, plants have to face and adapt to all kinds of environmental stresses. The most common biological damage to plants is caused by invasions of herbivores and pathogens. Infections by pathogens including bacteria, fungi, oomycetes and viruses, severely impair plant growth and are major factors detrimentally affecting crop production and food quality (Cook et al., 2015). Rice blast caused by the fungus *Magnaporthe oryzaecauses* costs about 6% of the annual rice production worldwide (Savary et al., 2019).

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Herbivores, such as insects and nematodes, damage plants by eating the vegetative tissues, fruits or seeds, and simultaneously spread a variety of diseases and thus reduce crop yields (Stokstad, 2017). For example, the fall armyworm (*Spodoptera frugiperda*), a highly polyphagous and widely migratory pest, has spread from the western hemisphere to more than 70 countries, resulting in the loss of 21%–53% of the annual corn production (Hussain et al., 2021). Moreover, indiscriminate use of synthetic pesticides, such as insecticides, attractants, nematicides and fungicides, usually comes with ecological and environmental consequences including pest resistance and environmental pollution (Ye et al., 2021). Therefore, it is essential and urgent to extend our understanding of the innate mechanisms of plant responses to biotic stresses, which provides the knowledge base to increase crop resistance to biotic stresses and ensure our food production without compromising our environment.

Plants have evolved a complicated and sophisticated recognition system to sense various environmental cues to initiate proper responses. For example, cell-surface pattern recognition receptors (PRRs) perceive biotic elicitors including pathogen-, microbe-, damage- and herbivore-associated molecular patterns (i.e., PAMPs, MAMPs, DAMPs and HAMPs, respectively) (Köster et al., 2022; Wang et al., 2022). For plant-microbe interactions, plants rely on two-layered and interconnected innate immune systems to initiate local response to pathogens. First, cell-surface localized PRRs perceive apoplastic immunogenic elicitors to activate pattern-triggered immunity (PTI) (Yu et al., 2017). Subsequently, intracellular nucleotide-binding leucine-rich repeat receptors (NLRs) recognize effectors secreted by pathogens to suppress PTI, thus initiating effector-triggered immunity (ETI), which is a process generally associated with hypersensitive response (HR) in the infection site (Zhou & Zhang, 2020). NLRs are classified into coiled-coil (CC)-NLRs (CNLs), toll/interleukin (TIR)-related-NLRs (TNLs), and RPW8 (CC_R)-NLRs (RNLs) according to the features of N-terminal domains. CNLs and TNLs function as sensors to identify effectors, while RNLs are reported to be helpers functioning together with TNLs (Feehan et al., 2020). Biotic stresses are also known to prime plant defense responses in neighbouring healthy cells away from the infection site, that is, the systemic acquired resistance (SAR) and the systemic wound response (SWR), thus providing long-lasting protection against a broad spectrum of pathogens and/or insect attacks (Hilleary & Gilroy, 2018). Before the establishment of plant immunity, biotic stresses are reported to induce numerous cellular signalling events in a rough temporal order, including Ca^{2+} flux, the production of reactive oxygen species (ROS), initiating mitogen-activated protein kinase (MAPK) cascade, transcriptional regulation, and the biosynthesis of defense-related phytohormones, such as salicylic acid (SA) and jasmonic acid (JA). In addition, some of these events are arranged in complicated feed-forward amplification loops and balancing signalling pathways (Köster et al., 2022), and Ca^{2+} transients are actively involved in many steps of plant immunity at different stages (Jiang & Ding, 2023) (Figure 1).

The transient changes of intracellular Ca^{2+} concentration triggered by various stimuli differ from each other in terms of amplitude, duration, frequency and spatial distribution inside the cell; these stimulus-specific Ca^{2+} transients are designated as Ca^{2+} signatures (Webb et al., 1996). It has been well-documented that Ca^{2+} signal is the key of plant immune system, and that changes in intracellular Ca^{2+} level are downstream to PRR and NLR activations (Moeder et al., 2019; Xu et al., 2022) (Figure 1). Flagellin Sensitive 2 (FLS2) and Elongation Factor Thermo Unstable (EF-Tu) Receptor (EFR), which sense bacterial PAMPs flg22 and elf18, respectively, are the best documented PRRs to date (Robatzek et al., 2006; Zipfel et al., 2006). FLS2 or EFR forms stable ligand-dependent complexes with the common co-receptor Brassinosteroid-insensitive 1-Associated Kinase 1 (BAK1, also named SERK3) that belongs to the Somatic Embryogenic Receptor Kinase (SERK) family (Heese et al., 2007; Roux et al., 2011). The complex formed by the combination of PRRs and coreceptors activates receptor-like cytoplasmic kinases (RLCKs) that directly phosphorylate Ca^{2+} channels, leading to the generation of cytoplasmic Ca^{2+} transients (Jeworutzki et al., 2010; Köster et al., 2022). On the other hand, recent studies revealed that NLRs activated by pathogen effectors can directly form noncanonical Ca^{2+} channels; recognition of effectors results in the oligomerization of plant NLRs, which then forms a protein complex with a wheel-like structure termed resistosomes. Pentameric CNL resistosomes, such as Hopz-activated Resistance 1 (ZAR1) from *Arabidopsis* and Stem Rust Resistance 35 (Sr35) from wheat (*Triticum aestivum L.*), function as noncanonical Ca^{2+} channels leading to the activation of immune signalling event of cell death (Bi et al., 2021; Förderer et al., 2022). Similarly, helper RNLs, Activated Disease Resistance 1 (ADR1) and N Requirement Gene 1.1 (NRG1.1), also form pentameric resistosomes and act as Ca^{2+} permeable channels to induce cell death (Jacob et al., 2021). CNL forms a channel downstream to TNL and the signal initiated is transduced via the involvement of Enhanced Disease Susceptibility 1 (EDS1). Tetramer TNL resistosomes usually act as NADase holoenzymes to generate secondary messengers (Horsefield et al., 2019; Ma et al., 2020), which are recognized by EDS1-Phytoalexin Deficient 4 (PAD4) and EDS1-Senescence-Associated Gene 101 (SAG101) complexes in *Arabidopsis* (Sun et al., 2021; Wu et al., 2021). The activated EDS1-PAD4 and EDS1-SAG101 complexes interact with and activate ADR1 and NRG1.1 (Dongus & Parker, 2021; Ma et al., 2020). Recently, a novel endoplasmic reticulum (ER)-localized Ca^{2+}-permeable channel named WeiTsing (WTS) was identified essential for clubroot resistance; it acts in the pericycle to prevent *Plasmodiophora brassicae* colonization in the stele in *Arabidopsis* (Wang, Qin, et al., 2023).

Cytoplasmic Ca^{2+} signal is sensed by various Ca^{2+}-binding proteins that act as Ca^{2+} sensors, which usually contain a conserved helix-loop-helix Ca^{2+} binding motif that is named 'EF-hand' and composed of 29 amino acids (Luan & Wang, 2021). Ca^{2+} binds to the Ca^{2+} sensor, resulting in conformational change of the EF-hand motif, thereby promoting the interaction of Ca^{2+} sensor with downstream proteins or regulates its own catalytic activity (Gifford et al., 2007). Usually, the Ca^{2+} sensors contain one or more EF-hand motifs, and

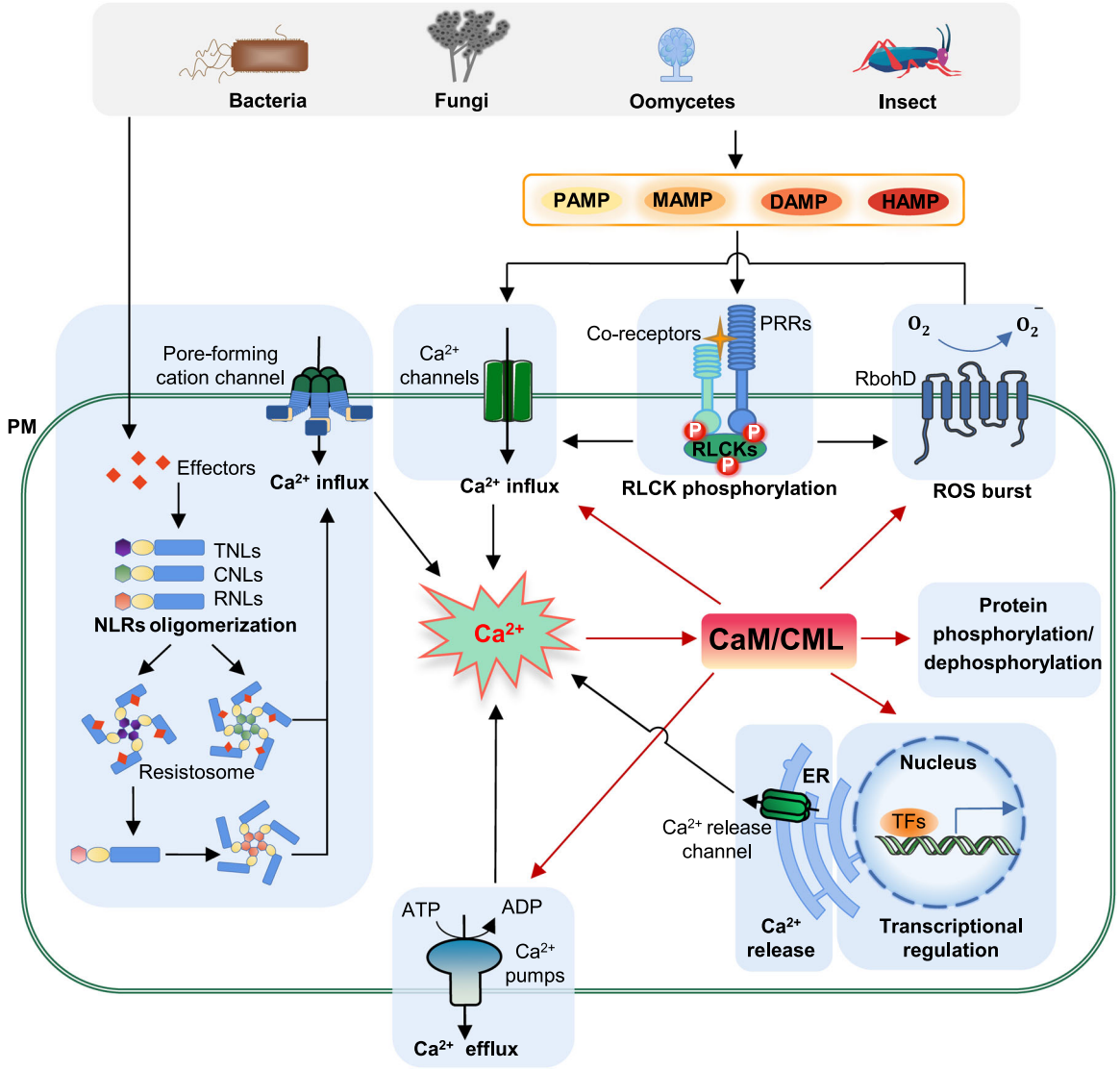

FIGURE 1 The generation of Ca^{2+} signals and the decoding of Ca^{2+} signals by CaMs/CMLs in plant responses to biotic stresses. Plants are exposed to pathogens, pests, or other aggressors, causing harmful biotic stresses. During pattern triggered immunity (PTI), pattern recognition receptors (PRRs) trigger its self-phosphorylation and subsequently phosphorylate targeted proteins including receptor-like cytoplasmic kinases (RLCKs). Phosphorylated RLCKs activate Ca^{2+} channels and/or Ca^{2+} pumps, leading to the dynamic change of cytosolic Ca^{2+} concentration, while activated RLCKs phosphorylate RBOHs and cause extracellular reactive oxygen species (ROS) accumulation, thus indirectly inducing Ca^{2+} influx in plant cells. Ca^{2+}-permeable channels located at endoplasmic reticulum (ER), such as WeiTsing, are also activated after pathogen infection to release Ca^{2+} into the cytoplasm for plant defenses. On the other hand, intracellular nucleotide-binding leucine-rich repeat receptors (NLRs) recognize effectors secreted by pathogens to activate a powerful immune response, called effector triggered immunity (ETI). The activated NLRs oligomerize on the plasma membrane (PM) and form an atypical Ca^{2+} permeation channel to induce Ca^{2+} influx. CaMs and CMLs, which decode Ca^{2+} transients, regulate the influx and efflux of Ca^{2+}, transcriptional regulation, and other cellular responses in response to biotic stresses. The red arrow indicates the decoding of Ca^{2+} signal by CaMs/CMLs. CNL, coiled-coil (CC)-NLR; DAMP, damage-associated molecular patterns; HAMP, herbivore-associated molecular patterns; MAMP, microbe-associated molecular patterns; PAMP, pathogen-associated molecular patterns; RNL, RPW8 (CC_R)-NLR; TNL, toll/interleukin (TIR)-related-NLR. [Color figure can be viewed at wileyonlinelibrary.com]

the sequence of EF-hand motifs is highly conserved (DeFalco et al., 2009). Calmodulins (CaMs) and CaM-like proteins (CMLs), Ca^{2+}-dependent protein kinases (CDPKs) and calcineurin B-like proteins (CBLs) are three major groups of EF-hand-containing Ca^{2+} sensors (DeFalco et al., 2009; Kudla et al., 2010). In this review, we summarized the roles of CaMs/CMLs and CaMs/CMLs-target proteins in regulating plant responses to biotic stresses.

2 | CAM/CML ACTING AS THE TRANSMITTER AND DECODER OF CA^{2+} SIGNAL

CaM, a small acidic protein showing a dumbbell-shaped structure when loaded with Ca^{2+}, is ubiquitous and highly conserved in eukaryotes (DeFalco et al., 2009; Mohanta et al., 2017). CaM is

composed of a flexible central linker connecting two roughly symmetrical spherical domains located at the N- and C-terminus; each domain contains a pair of EF-hand motifs and in other words, one CaM possesses four Ca^{2+} binding sites, with two relatively low-affinity sites located in the N-terminus and two high-affinity sites located in the C-terminus (Bouché et al., 2005). Upon Ca^{2+} binding, the conformation of CaM changes from a closed and Ca^{2+}-free state (apo-CaM) to an extended conformation (Ca^{2+}-loaded CaM), leading to a transition from a hydrophilic state to a predominantly hydrophobic state on the protein surface (La Verde et al., 2018; Poovaiah et al., 2013). In addition to canonic CaMs, plants also possess CMLs that share similar structures with CaMs, but CMLs generally possess one to six EF-hand motifs (Mohanta et al., 2017; Zhu et al., 2015). Despite sequence divergence of EF-hand motifs, experiments by gel mobility shift assay, isothermal titration calorimetry (ITC), and/or nuclear magnetic resonance (NMR) show that CMLs could bind to Ca^{2+} through EF-hand motifs, indicating that CMLs also act as Ca^{2+} sensors (Dobney et al., 2009; Vanderbeld & Snedden, 2007; Zeng et al., 2017). However, CMLs exhibit structural and bio-functional difference from CaMs. Unlike CaMs, not all EF-hand motifs in CMLs possess Ca^{2+}-binding ability. For example, ITC and NMR revealed that only one of three EF-hand motifs in AtCML14 binds Ca^{2+} (Vallone et al., 2016); both AtCML15 and AtCML16 contain four predicted EF-hand motifs, of which only two and three show Ca^{2+}-binding ability, respectively (Ogunrinde et al., 2017). In addition, the Ca^{2+} affinity in CMLs is substantially different from that in CaMs, suggesting that they could be activated differentially at distinct Ca^{2+} concentrations during Ca^{2+} oscillation. In structure, CMLs are relatively variant in length and contain an N- or C-terminal extension in comparison to CaMs (Figure 2a); this may lead to a different conformational allostery when binding with their target proteins. Moreover, the different length and low sequence similarity of the linker regions between CaMs and CMLs could result in a significant difference in the flexibility of CMLs and their ability to interact with target proteins (La Verde et al., 2018). For example, regulator of gene silencing CaM (rgs-CaM), a unique CML in tobacco, interacts with its targets via electrostatic interaction, but not via hydrophobic interaction, which is a canonical CaM-target binding mechanism (Makiyama et al., 2016).

At present, many *CaM* and *CML* genes have been identified in diverse plant species. For example, there are seven *CaM* genes encoding four proteins (CaM1/4, CaM2/3/5, CaM6 and CaM7) and 50 *CML* genes in *Arabidopsis thaliana* (McCormack & Braam, 2003); there are 5 *CaM* genes and 32 *CML* genes in rice (*Oryza sativa*) (Boonburapong & Buaboocha, 2007); there are 6 *CaM* genes and 144 *CML* genes in soybean (*Glycine max*)(Zeng et al., 2017); 6 *CaM* genes and 52 *CML* genes exist in tomato (*Solanum lycopersicum*) (Munir et al., 2016; Zhao et al., 2013), and 18 *CaM* genes and 230 *CML* genes exist in wheat (Liu et al., 2022). To date, except for *Arabidopsis* CaM7, which was reported to specifically bind to the Z-/G-box in a Ca^{2+}-dependent manner and act as a transcription factor (TF) to regulate the expression of light-responsive genes (Abbas et al., 2014; Kumar et al., 2016), all other CaMs and CMLs are believed to lack enzymatic or transcription-activation activity and function by interacting with and regulating their target proteins. Different CaM isoforms differ in their recognition of target proteins (Gifford et al., 2013; Lee et al., 2000; Yoo et al., 2005), indicating the slight difference in their structural features may have considerable impacts when they bind to target proteins (La Verde et al., 2018; Yamniuk & Vogel, 2005). Therefore, the identification of CaM/CML target proteins, also known as CaM-binding proteins (CBPs), and the understanding of the functions of their target proteins is vital to explore the role of CaM/CML-mediated signalling pathways in plant responses to biotic stresses.

3 | INVOLVEMENT OF CAMS/CMLS IN COPING WITH BIOTIC STRESSES

CaM isoforms from diverse plant species exhibit differential expression patterns in response to biotic stimuli (Yamakawa et al., 2001) (Table 1). *GmCaM4* and *GmCaM5*, two divergent CaM isoforms in soybean, are rapidly induced by fungal elicitors (Heo et al., 1999). Overexpression of *GmCaM4* or *GmCaM5* in tobacco, *Arabidopsis* and soybean triggers the spontaneous lesions, induces a series of SAR-related genes, and enhances the broad-spectrum resistance to a variety of pathogens, such as the oomycete *Phytophthora sojae* and two necrotrophic fungal pathogens, *Alternaria tenuissima* and *Phomopsis longicolla* (Heo et al., 1999; Park et al., 2004; Rao et al., 2014). Silencing the pathogen-induced *NtCaM13*, a homologous gene of *GmCaM4* and *GmCaM5*, resulted in elevated susceptibility to virulent necrotrophic pathogens in tobacco, suggesting its positive role in the basal defense against necrotrophic pathogens (Takabatake et al., 2007). Pepper (*Capsicum annuum*) CaCaM1 was suggested to be involved in the production of ROS and nitric oxide (NO) that are involved in regulating cell death and defense responses (Choi et al., 2009). In tomato, silencing of *SlCaM2* and *SlCaM6* alters the expression of a set of defense-related genes and results in dramatically reduced resistance to tobacco rattle virus (TRV), and the oomycete pathogen *Pythium aphanidermatum* (Zhao et al., 2013).

APR134 encoding a CML in tomato and APR134-like protein *CML43* in *Arabidopsis* are both induced by pathogens; silencing of *APR134* by virus-induced gene silencing results in reduced resistance to pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst* DC3000), while overexpression of *CML43* promotes pathogen-triggered HR in *Arabidopsis* (Chiasson et al., 2005). *Arabidopsis* CML8 and CML9 belonging to the same CML subgroup have been identified as positive regulators of defense responses against *Pst* DC3000 (Leba et al., 2012; Zhu et al., 2017). *Arabidopsis* CML24 also acts as a positive regulator in plant immunity based on the evidence that loss-of-function mutant *cml24* displayed an impaired innate immune response and decreased PAMP-triggered NO production (Ma et al., 2008). *Arabidopsis* CML41 is a plasmodesmata-located Ca^{2+}-responsive protein that mediates

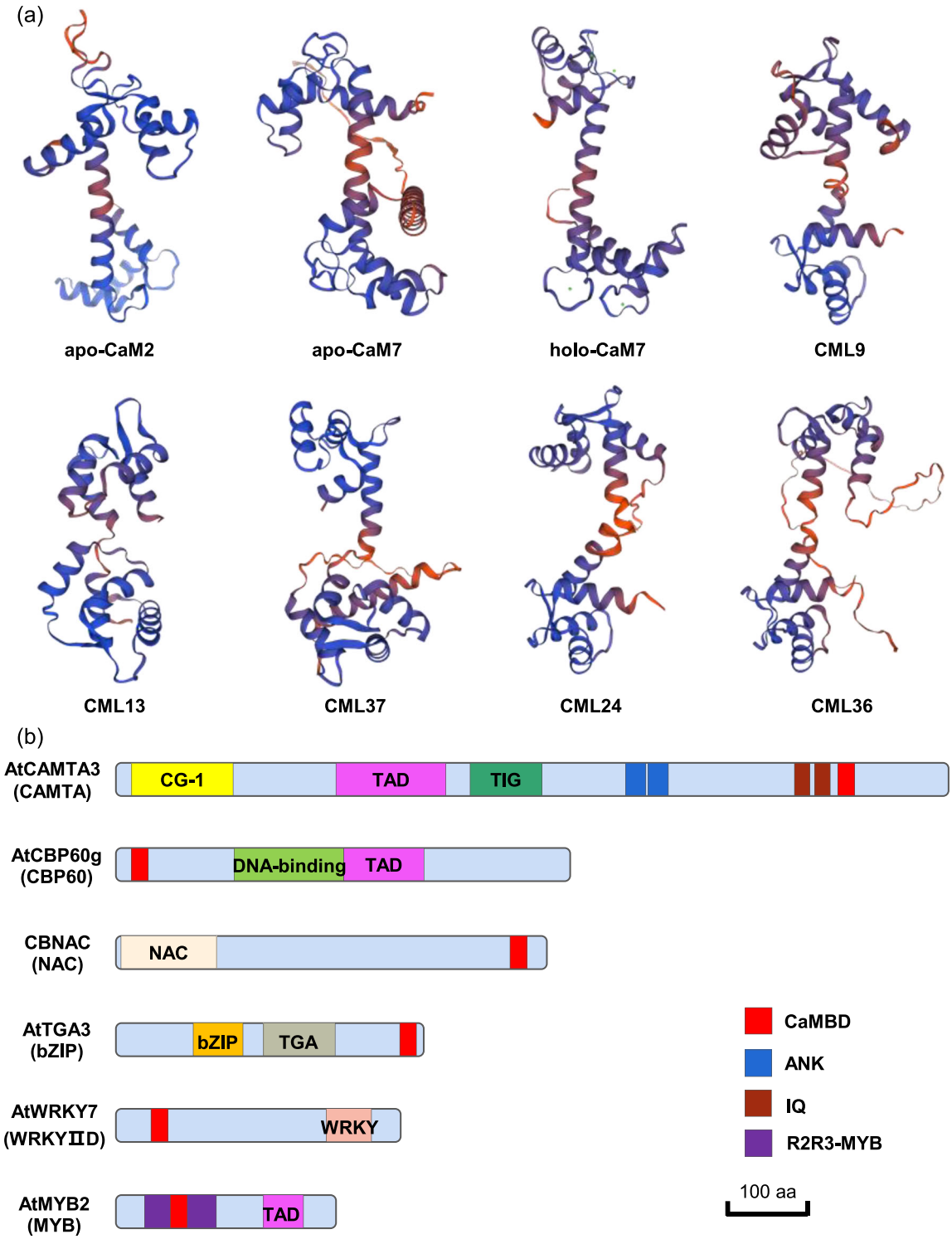

FIGURE 2 Structures of representative CaMs/CMLs and structural organization of typical CaM-binding transcription factors in *Arabidopsis*. (a) Predicted ribbon models of CaMs and CMLs in the apo- and Ca^{2+} bound (holo-) form were generated using the SWISS-MODEL (https://swissmodel.expasy.org/). (b) Schematic representation of the structural organization of six typical CaM-binding transcription factors in *Arabidopsis*. Functional protein domains of each transcription factors are shown. The name in the bracket is the family name of the transcription factor. ANK, anchor protein repeat domain; ATAF, CUC (cup-shaped cotyledon); CaMBD, calmodulin binding domain; NAC, NAM (no apical meristem); TAD, transcriptional activation domain; TIG, transcription factor immunoglobulin domain.

TABLE 1 Involvement of CaMs, CMLs and CBPs in plant responses to biotic stresses.

Gene name	Plant species	Roles in biotic stress response	Subcellular localization	References
CaM				
CaCaM1	*Capsicum annuum*	*Xanthomonas campestris* pv. *Vesicatoria* Ds1 (bacteria) ↑	Cytosolic and PM	Choi et al. (2009)
	Arabidopsis thaliana	*Pseudomonas syringae* pv. tomato DC3000 (bacteria) ↑ *Hyaloperonospora parasitica* Noco2 (oomycete) ↑ *Alternaria brassicicola* KACC40036(fungi) ↓		
AtCaM1	*Arabidopsis thaliana*	*Spodoptera littoralis* (herbivory) ↓	–	Jiao, Li, et al. (2022)
SlCaM2	*Solanum lycopersicum*	Tobacco rattle virus virus) ↑ *Pythium aphanidermatum* (oomycete) ↑	–	Peng et al. (2014); Zhao et al. (2013)
SlCaM6		Tobacco rattle virus (virus) ↑ *Pythium aphanidermatum* (oomycete) ↑ *Botrytis cinerea* 22B (fungi) ↑	–	
CML				
GmCaM4	*Glycine max*	*Phytophthora sojae* race 3 (oomycete) ↑ *Alternaria tenuissima* (fungi) ↑ *Phomopsis longicolla* (fungi) ↑	Nucleus and cytoplasm	Heo et al. (1999); Rao et al. (2014)
	Nicotiana tabacum	*Phytophthora parasitica* var. *nicotianae* (fungi) ↑ *Pseudomonas syringae* pv. *tabaci* (bacteria) ↑ Tobacco mosaic virus (virus) ↑		
GmCaM5	*Nicotiana tabacum*	*Phytophthora parasitica* var. *nicotianae* (fungi) ↑ *Pseudomonas syringae* pv. *tabaci* (bacteria) ↑ Tobacco mosaic virus (virus) ↑	–	
NtCaM13	*Nicotiana tabacum*	Tobacco mosaic virus (virus) ↑ *Ralstonia solanacearum* 8225 (bacteria) ↑ *Rhizoctonia solani* (fungi) ↑ *Pythium aphanidermatum*(fungi) ↑	–	Takabatake et al. (2007); Yamakawa et al. (2001)
rgs-CaM	*Nicotiana tabacum*	Cucumber mosaic virus (virus) ↑ Tobacco etch virus (virus) ↑	–	Jeon et al. (2017); Nakahara et al. (2012)
SlAPR134	*Solanum lycopersicum*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↑	–	Chiasson et al. (2005)
AtCML43	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↑	–	Chiasson et al. (2005)
AtCML24	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↑	–	Ma et al. (2008)
AtCML9	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↑	–	Leba et al. (2012)
AtCML8	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↑	–	Zhu et al. (2017)
AtCML41	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↑	Plasmodesmata	Xu et al. (2017)
AtCML46	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *maculicola* ES4326 (bacteria) ↑	–	Lu et al. (2018)
AtCML47			–	
SlCML55	*Solanum lycopersicum*	*Phytophthora capsici* (oomycete) ↓	Cytosol and nucleus	Zhang et al. (2022)

TABLE 1 (Continued)

Gene name	Plant species	Roles in biotic stress response	Subcellular localization	References
TaCML36	*Triticum aestivum*	*Rhizoctonia cerealis* (fungi) ↑	Cytoplasm and nucleus	Lu et al. (2019)
CaCML13	*Capsicum annuum*	*Ralstonia solanacearum*(bacteria) ↑	—	Shen et al. (2020)
GhCML11	*Gossypium hirsutum*	*Verticillium dahliae* (fungi) ↑	Apoplast, nucleus and cytoplasm	Cheng et al. (2016)
AtCML42	*Arabidopsis thaliana*	*Spodoptera littoralis* (herbivore) ↓ *Alternaria brassicicola* (fungi) ↓	Cytosol and nucleus	Heyer et al. (2022); Vadassery et al. (2012)
AtCML37	*Arabidopsis thaliana*	*Spodoptera littoralis* (herbivory) ↑ *Alternaria brassicicola* (fungi) ↑	Cytosol and nucleus	Heyer et al. (2022); Scholz et al. (2014)
CBP (transcription factor and co-factor)				
AtCBP60g	*Arabidopsis thaliana*	*Pseudomonas syringae pv. tomato* DC3000 (bacteria) ↑ *Pseudomonas syringae pv. maculicola* ES4326 (bacteria) ↑	Nucleus	Wan et al. (2012); Wang et al. (2009); Zhang et al. (2010)
SARD1	*Arabidopsis thaliana*	*Hyaloperonospora arabidopsidis* Noco2 (oomycete) ↑ *Pseudomonas syringae* pv. *maculicola* ES4326 (bacteria) ↑	Nucleus	Zhang et al. (2010)
AtCBP60b	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↑	Nucleus	Huang et al. (2021); Li et al. (2021)
GhCBP60b	*Gossypium hirsutum*	*Verticillium dahlia* V592 (fungi) ↑	–	Qin et al. (2018)
AtCBP60a	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *maculicola*ES4326(bacteria)↓ *Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	–	Truman et al. (2013)
AtCAMTA3	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓ *Sclerotinia sclerotiorum*(fungi) ↓ *Botrytis cinerea* B05.10 (fungi) ↓ *Bradysia impatiens* (herbivore) ↑	Nucleus	Du et al. (2009); Galon et al. (2008); Qiu et al. (2012); Rahman et al. (2016); Yuan et al. (2021)
OsCBT	*Oryza sativa*	*Magnaporthe grisea* KJ401/KI313 (fungi) ↓ *Xanthomonas oryzae* pv. *oryzae* K1/K2(bacteria) ↓	–	Koo et al. (2009)
SlSR1 SlSR3L	*Solanum lycopersicum*	*Botrytis cinerea* (fungi) ↓ *Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	Nucleus	Li et al. (2014)
TaCAMTA4	*Triticum aestivum*	*Puccinia triticina* ↓	Cytoplasm and nucleus	Wang et al. (2019)
AtWRKY11 AtWRKY17	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	– –	Journot-Catalino et al. (2006)
AtWRKY7	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	Nucleus	Kim et al. (2006)
AtWRKY53	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* (bacteria) ↑ *Spodoptera littoralis* (herbivore) ↓	–	Jiao, Li, et al. (2022); Murray et al. (2007)
AtTGA3	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *maculicola* ES4326 (bacteria) ↑	–	Kesarwani et al. (2007)
CBNAC	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	–	Kim et al. (2012)

(Continues)

TABLE 1 (Continued)

Gene name	Plant species	Roles in biotic stress response	Subcellular localization	References
CBP (transporter)				
AtCNGC2	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	PM	Ali et al. (2007); Clough et al. (2000)
AtCNGC4	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	PM	Balague et al. (2003); Tian et al. (2019)
AtCNGC11 AtCNGC12	*Arabidopsis thaliana*	*Hyaloperonospora parasitica* (oomycete) ↑ *Pseudomonas syringae pv. maculicola* ES4326 (bacteria) ↑	PM	Urquhart et al. (2007); Yoshioka et al. (2006)
AtCNGC19	*Arabidopsis thaliana*	Cell death *Spodoptera litura* (herbivory) ↑	PM	Meena et al. (2019); Yu et al. (2019)
AtCNGC20	*Arabidopsis thaliana*	Cell death *Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↑	PM	Yu et al. (2019); Zhao et al. (2021)
AtACA4 AtACA11	*Arabidopsis thaliana*	Cell death	Tonoplast	Boursiac et al. (2010)
AtACA8 AtACA10	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	PM	Yang et al. (2017)
NbCA1	*Nicotiana benthamiana*	Cell death	ER	Zhu et al. (2010)
HvMLO	*Hordeum vulgare*	*Blumeria graminis* f. sp. *hordei* (fungi) ↓ *Bipolaris sorokiniana* (fungi) ↑ *Magnaporthe grisea* race 007 (fungi) ↑	PM	Jarosch et al. (1999); Kim, Panstruga, et al. (2002); Kumar et al. (2001)
AtPEN3	*Arabidopsis thaliana*	*Plectosphaerella cucumerina* (fungi) ↑ *Erysiphe pisi* (fungi) ↑ *Phytophthora infestans* (fungi) ↑	PM	Campe et al. (2016)
CBP (protein phosphatase)				
AtMKP1	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓	Cytoplasm	Anderson et al. (2011); Bartels et al. (2009)
OsMKP1	*Oryza sativa*	Wound ↓	–	Katou et al. (2007)
NtMKP1	*Nicotiana tabacum*	Wound ↓ *Botrytis cinerea* (fungi) ↓ *Mamestra brassicae* (herbivore) ↓ *Spodoptera litura.* (herbivore) ↓	–	Oka et al. (2013); Yamakawa et al. (2004)
CBP (protein kinase)				
AtMPK8	*Arabidopsis thaliana*	Wound-induced ROS accumulation ↓	–	Takahashi et al. (2011)
CBP (metabolic enzyme)				
NtGAD	*Nicotiana tabacum*	*Heliothis virescens* (herbivore) ↑ *Meloidogyne hapla* (herbivore) ↑	–	MacGregor et al. (2003); McLean et al. (2003)
AtGAD4	*Arabidopsis thaliana*	*Plutella xylostella* (herbivory) ↑	–	Jiao, Guo, et al. (2022)
CBP (other protein)				
AtIQD1	*Arabidopsis thaliana*	*Myzus persicae* (herbivory) ↑ *Trichoplusiani* (herbivory) ↑ *Botrytis cinerea* (fungi) ↑	Nucleus	Barda & Levy (2022); Levy et al. (2005)
CaMLO2	*Capsicum annuum*	*Xanthomonas campestris* pv. *vesicatoria* (bacteria) ↓	PM	D. H. Kim & Hwang (2012)

TABLE 1 (Continued)

Gene name	Plant species	Roles in biotic stress response	Subcellular localization	References
	Arabidopsis thaliana	*Pseudomonas syringae* pv. *tomato* DC3000 (bacteria) ↓ *Hyaloperonospora arabidopsidis* Noco2 (oomycete) ↓		
CsMLO1	*Cucumis sativus L.*	*Corynespora cassiicola* (fungi) ↓	PM	Yu et al. (2019)
AtBAG6	*Arabidopsis thaliana*	*Botrytis cinerea* (fungi) ↑	Nucleus	Kang et al. (2006); Li et al. (2016)

Note: "↑" denotes a positive role in defense, "↓" denotes a negative role in defense, and "–" denotes unknown.
Abbreviations: ER, endoplasmic reticulum; PM, plasma membrane.

flg22-induced callose-dependent plasmodesma closure and positively regulates defense against *Pst* DC3000 (Xu et al., 2017).

By contrast, some CMLs negatively regulate plant defense responses. Both CML46 and CML47 in *Arabidopsis* negatively regulate plant immunity, as the double mutant *cml46 cml47* is highly resistant to *P. syringae* pv. maculicola and is impaired in the repression of immune responses in the absence of pathogens (Lu et al., 2018). CMLs in other plant species also play a key role in plant defense against pathogens. For example, wheat TaCML36 positively regulate plant immune responses to soil-borne fungus *Rhizoctonia cerealis* (Lu et al., 2019). Pepper CaCML13 acts as a positive regulator in immunity against *Ralstonia solanacearum* (Shen et al., 2020). Cotton GhCML11 interacts with GhMYB108 to initiate plant defense against the infection of pathogenic fungus *Verticillium dahliae* (Cheng et al., 2016). Tomato SlCML55 acts as a negative regulator of defense by suppressing SA signalling pathway (Zhang et al., 2022). In addition, a CML named rgs-CaM is involved in the regulation of virus-induced RNA silencing by directly interacting with virus RNA silencing suppressors (Nakahara et al., 2012). Overexpression of *rgs-CaM* enhances defense responses, including generation of high level of ROS, activated SA signalling and increased cell death, and thus confers resistance to cucumber mosaic virus (CMV) (Jeon et al., 2017).

CaMs/CMLs are also involved in plant defense against herbivory. *Arabidopsis* CML37 is induced by mechanical injury and infestation with larvae of the generalist lepidopteran herbivore, and positively regulates the defense response to cotton leafworm (*Spodoptera littoralis*) by activating JA pathway (Scholz et al., 2014). CML42 negatively regulates plant response to cotton leafworm by decreasing JA sensitivity and the expression of JA-responsive genes in *Arabidopsis* (Vadassery et al., 2012). Besides involvement in the defense against herbivores, JA is also an important hormone in plant immune response to necrotrophic pathogen infections. In coping with necrotrophic pathogen *Alternaria brassicicola*, *cml37* mutant is more sensitive, but *cml42* is more resistant than wild-type, while *cml37 cml42* double mutant shows a wild-type phenotype, suggesting antagonistic roles of these two CMLs (Heyer et al., 2022). Apparently, CML42 and CML37 act as Ca^{2+} sensors and coordinate Ca^{2+} signals and JA-mediated defense to herbivores and necrotrophic pathogens.

4 | CAM-BINDING PROTEINS: DOWNSTREAM TARGETS OF CAMS/CMLS

CaMs/CMLs, acting as sensors and transmitters of Ca^{2+} signal in plant cells, regulate the functions of a large group of downstream CBPs by forming complexes in a Ca^{2+}-dependent or independent manner, and the latter is usually involved in a Ca^{2+}-mediated disruption of the interacting complex (Reddy et al., 2011; Zeng et al., 2015) (Table 1). Hence, identifying and characterizing the physiological functions of CBPs provide reliable and practical approaches to address the functionalities of CaMs/CMLs. The CaM-binding domain (CaMBD, usually containing 16-35 amino acids) in CBPs, is necessary for its interaction with CaMs or CMLs in a Ca^{2+}-dependent manner (Bouché et al., 2005). CaMBD is not conserved in the amino acid sequence, but has a similar secondary structure, i.e., a basic amphipathic helix with hydrophobic residues arranged on one side and positively charged residues arranged on the other side. Some CBPs also carry the IQ motif ([I/L/V] QxxxRGxxx [R/K]), which interacts with CaM in a Ca^{2+}-dependent manner or Ca^{2+}-independent manner (Bähler & Rhoads, 2002). The regulation of CaM over its target proteins occurs in a variety of ways, such as relieving the automatic inhibition of active sites, dimerization, scaffolding and remodelling (Bouché et al., 2005). Like CaMs, CMLs also interact with CBPs, although the molecular mechanism in detail is still unclear. The conventional methods for detecting protein-protein interactions, such as yeast two-hybrid and co-immunoprecipitation, are ineffective in identifying Ca^{2+}-dependent interaction between CaMs/CMLs and their target proteins. Most CBPs in plants have been identified by screening cDNA expression libraries with labelled CaM as a probe, mostly ^{35}S-labeld CaM or horseradish peroxidase (HRP)-CaM (Fromm & Chua, 1992; Yang & Poovaiah, 2000b). Another effective method to identify CBPs is using protein microarrays (Popescu et al., 2007), but false positive identification is still an obvious problem, and also it is a challenge to make protein chips with sufficient coverage. In the past decades, the development of large-scale proteome analysis has increased the understanding of CBPs. CaM/CML plays a regulatory role in a variety of cellular processes by regulating the activities of various proteins, including kinases, phosphatases, metabolic enzymes, TFs, ion channels/transporters, heat shock proteins and proteins with unknown functions (Bouché et al., 2005; Reddy et al., 2011; Zeng et al., 2015) (Table 1). Therefore, it can be reasonably concluded that in most cases, CaMs and

CMLs acting as Ca^{2+} signal decoders are multifunctional regulatory proteins, and their functional significance is dependent on the particular role of their target proteins.

4.1 | CaM/CML-mediated transcriptional regulation under biotic stresses

Rapid transcriptional reprogramming is crucial for plants to survive biotic stresses. Many TFs have been identified as CBPs. For example, CBP60, CAMTA, and WRKY IID families are representative CaM-binding TFs involved in plant responses to biotic stresses (Figure 2b). Here, we summarized roles of the major CaM-binding TFs implicated in the regulation of plant responses to biotic stresses (Table 1, Figure 3).

4.1.1 | CBP60 acting as CaM-binding TFs

Calmodulin-binding protein 60 (CBP60) family is a typical TF family in plants, which was first isolated from maize (*Zea mays*) (Reddy et al., 1993). Among the eight members of the CBP60 family (CBP60a-g and SARD1) in *Arabidopsis*, CBP60a to CBP60f contain CaMBD at the C-terminus, and CBP60g contains one at the N-terminus, while SARD1 lacks CaMBD and thus cannot be bound by CaM (Reddy et al., 2002; Wang et al., 2009, 2011). *CBP60g* is the first *CBP60* family gene reported to be induced by *Pst* DC3000 and involved in plant immune responses (Wang et al., 2009). Complementation of mutated CBP60g with abolished CaM-binding capability, failed to rescue the phenotype of *cbp60* null mutant, indicating that CaM-binding capability is required for the normal function of CBP60g (Wang et al., 2009).

While searching for mutants with defects in SAR, SAR-deficient 1 (SARD1) was identified to be the eighth member of CBP60 family displaying partially functional redundancy with CBP60g (Zhang et al., 2010). A single knockout mutant of SARD1 or CBP60g shows decreased SA production and compromised disease resistance (Zhang et al., 2010). In *sard1cbp60g* double mutant, SA biosynthesis is significantly reduced in local and distal leaves, leading to impairment of basic resistance and loss of SAR (Wang et al., 2009; Zhang et al., 2010). Both SARD1 and CBP60g specifically bind to

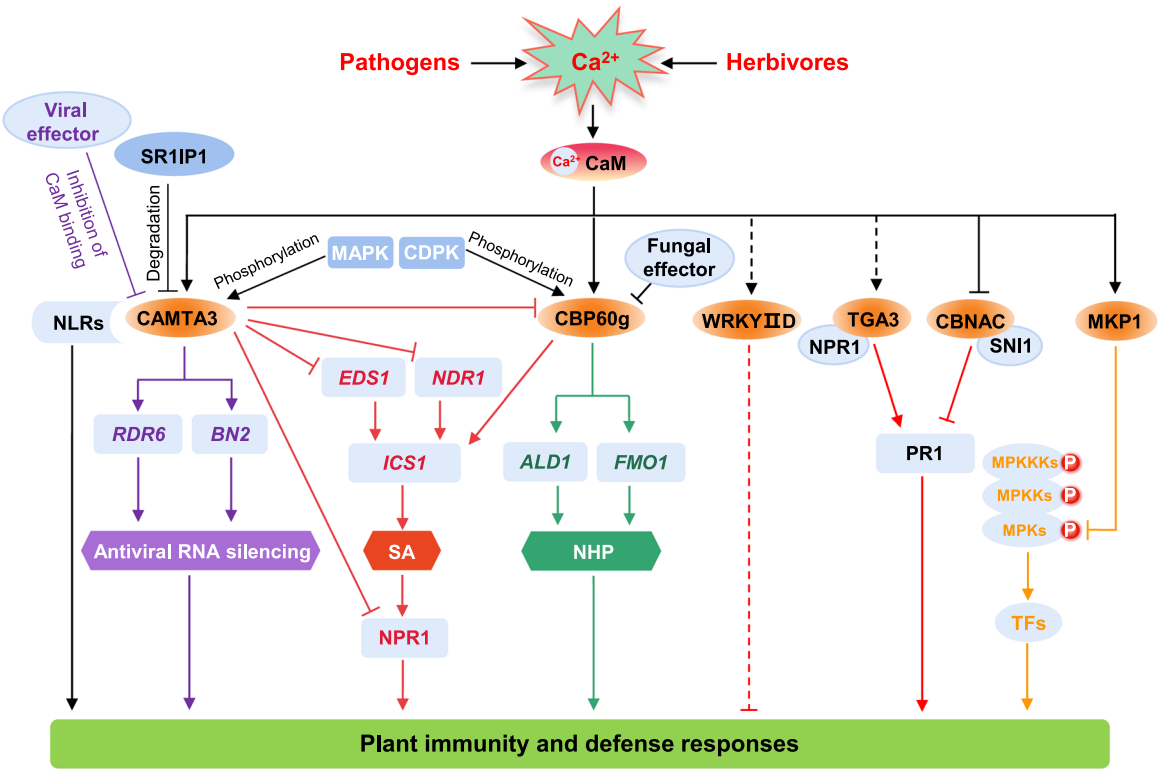

FIGURE 3 CaM/CML-mediated transcriptional regulation in plant responses to biotic stresses. The Ca^{2+} signal induced by biotic stresses is decoded by CaMs/CMLs, which then interact with CaM-binding transcription factors for transcriptional regulation. CAMTA3 negatively regulates SA signalling pathway and positively regulates plant antiviral activity. CAMTA3 also functions as a target protein of virus-derived effector, and is guarded by NLRs. Phosphorylation of CAMTA3 by CDPKs and MAPKs facilitates the nuclear export and thus activates the immune response. CBP60g is involved in plant immunity by regulating the expression of genes associated with biosynthesis of SA and NHP. Other CaM-binding transcription factors, such as CBNAC and TGA3, play a key role in the expression of the SA responsive gene *PR1*. It is worth noting that pathogen invasion leads to the activation of MAPK cascade, while MKP1, acting as a CBP, inhibits MAPKs. The orange ovals represent CBPs. Arrows head designate activation of gene expression; Lines with 'T' head designate suppression. Dashed lines designate hypothetical pathways. [Color figure can be viewed at wileyonlinelibrary.com]

"GAAATTTGG" motif in the promoter of *Isochorismate Synthase 1 (ICS1)*, which encodes a critical enzyme for SA biosynthesis, suggesting that SA biosynthesis could be activated through both Ca^{2+}/CaM-dependent and independent pathways (Zhang et al., 2010). In addition, SARD1 and CBP60g directly regulate the expression of *AGD2 like Defense Response Protein 1* (*ALD1*) and *Flavin Dependent Monooxygenase 1* (*FMO1*), both of them encoding key catalytic enzymes in the biosynthesis of pipecolic acid (Pip) and N-hydroxypipecolic acid (NHP); Pip and NHP are indispensable signal molecules for SAR (Jiang et al., 2021; Sun et al., 2015).

In addition, *Arabidopsis* CBP60g is involved in the host resistance to *Verticillium dahliae*. Recent studies indicate that the *V. dahliae* secretes an intracellular effector VdSCP41 that directly targets CBP60g and interferes its activity to inhibit plant immunity and promote pathogenicity, suggesting that Ca^{2+}/CaM signalling plays a key role in plant immunity to fungal pathogens (Qin et al., 2018). It has also been found that PAMPs derived from *V. dahliae* activate the immune response by inducing the phosphorylation of CBP60g. Touch 3 (TCH3), a CML protein, binds to CPK5, and promotes the CPK5-mediated phosphorylation of CBP60g to regulate plant resistance to *V. dahlia* (Sun et al., 2022). Therefore, Ca^{2+}/CaM exerts multiple regulations over CBP60g at both transcriptional and posttranslational levels during plant immunity (Figure 3).

In contrast to CBP60g and SARD1, CBP60a negatively regulates plant immunity (Truman et al., 2013). Basal SA and *ICS1* expression levels are increased in *cbp60a* mutant, suggesting that CBP60a could either directly or indirectly regulate the expression of *ICS1* and SA biosynthesis. CBP60a binds CaM with the CaMBD located at the C-terminal, and the CaM binding is essential for the immunity-repressing function of CBP60a (Truman et al., 2013). CBP60b positively regulates plant immune activation by directly targeting the promoter of *SARD1* (Huang et al., 2021). Plants overexpressing *CBP60b* display phenotypes of a constitutively enhanced immune responses (Huang et al., 2021; Li et al., 2021). Interestingly, *cbp60b* knockout mutant displays an EDS1-dependent autoimmune phenotype, suggesting that CBP60b is involved in TNL-mediated immunity (Huang et al., 2021; Li et al., 2021). CBP60 homologues in other plants, such as *PvCBP60-C* and *PvCBP60-D* in common bean (*Phaseolus vulgaris*) and *GhCBP60b* in cotton (*Gossypium hirsutum*), are also involved in plant immune responses (Ali et al., 2003; Qin et al., 2018), suggesting their conserved roles in higher plants.

4.1.2 | CAMTAs/SRs

Calmodulin-binding transcription activators (CAMTAs), also known as signal-responsive (SR) proteins, exist in all multicellular organisms (Wang et al., 2015; Xiao et al., 2021). CAMTA was first identified to be a Ca^{2+}/CaM-binding TF in tobacco, which is named ethylene-responsive 1 (NtER1) (Yang & Poovaiah, 2000a). In *Arabidopsis*, loss-of-function mutant *camta3/sr1* shows an autoimmune phenotype, enhanced SA accumulation and increased resistance against multiple pathogens including bacterial pathogen *Pst* DC3000 (Du et al., 2009), and fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea* (Galon et al., 2008; Rahman et al., 2016), suggesting its negative role in plant immunity. It has been well-documented that CAMTA3 negatively regulates the expression of several important immunity-related genes by binding to the CG-box cis-regulatory element ((A/C/G)CGCG(T/C/G)) in promoter regions of its target genes (Yang & Poovaiah, 2002a), thereby fine-tuning SA biosynthesis, SA signalling, and immune responses (Figure 3). The target genes of CAMTA3 include *EDS1*, which encodes a component downstream of TNLs (Du et al., 2009), *Non-race-specific Disease Resistance 1 (NDR1)*, which encodes a key component in CNL-mediated plant immunity (Nie et al., 2012), *Non-expressor of Pathogenesis-related Proteins 1* (*NPR1*), which encodes an SA receptor (Yuan et al., 2021), and *CBP60g*, which positively regulates SA biosynthesis (Sun et al., 2020). Notably, CAMTA1, CAMTA2 and CAMTA3 have redundant functions in plant immunity, but the single mutants of *CAMTA1* and *CAMTA2* do not have phenotypes related to defense responses (Sun et al., 2020). Upon pathogen infections, the activities of CAMTA1/2/3 are inhibited and expressions of their downstream target genes, *CBP60g* and *SARD1*, are activated, and subsequently the expressions of *ICS1*, *ALD1* and *FMO1* that are regulated by CBP60g and SARD1 are induced, thus promoting the biosynthesis of SA, Pip and NHP, and further activating local and systemic defense responses (Kim et al., 2020; Sun et al., 2020) (Figure 3). In addition, CAMTAs in other plants species were also found to negatively regulate plant defenses. For example, *Oryza sativa* CaM-binding transcription factor (OsCBT) was identified to be a negative regulator in rice resistance to both bacterial pathogen *Xanthomonas oryzae* and the rice blast pathogenic fungus *Magnaporthe grisea* (Choi et al., 2005; Koo et al., 2009). SlSR3L and SlSR1 in tomato, and TaCAMTA4 in wheat also play negative role in defense against pathogens (Li et al., 2014; Wang et al., 2019).

Since CAMTA3 acts as a suppressor of SA-mediated defense response, mechanisms must have been evolved for plants to overcome this suppression and mount effective defense in response to pathogen attacks. Two different molecular mechanisms have been known to eliminate CAMTA3-mediated suppression of plant immune response, the nuclear export and protein degradation of CAMTA3. Two flg22-responsive MAPKs, MPK3 and MPK6, directly phosphorylate CAMTA3, and contribute to nuclear export and degradation of CAMTA3, which further derepresses the expression of CAMTA3-targeted genes (Jiang et al., 2020). CDPKs may also participate in pathogen-induced phosphorylation of CAMTA3 in an indirect way (Jiang et al., 2020). The ubiquitin/proteasome-mediated degradation of CAMTA3 involves SR1 Interacting Protein 1 (SR1IP1), which is a substrate adaptor of ubiquitin ligase (Zhang et al., 2014). In addition, CAMTA3 is guarded by two NLR proteins, that is, Dominant Suppressor of *camta3* 1 (DSC1) and DSC2 (Lolle et al., 2017). However, whether the guard of CAMTA3 by NLRs is associated with the subcellular localization and posttranslational modification of CAMTA3 is still unclear.

CAMTA3 also plays a positive role in RNA interference (RNAi) mediated immune response against viral infection in *N. benthamiana* (Wang et al., 2021). CAMTA3 binds to the promoters of *RNA-dependent RNA polymerase 6 (RDR6)* and *Bifunctional Nuclease 2 (BN2)*

to induce their expression. RDR6 catalyzes the conversion of single-stranded RNA into double-stranded RNA to facilitate RNAi, while *BN2*, a novel RNAi-related gene encoding a ribonuclease, activates the expression of key genes in the RNAi pathway by degrading microRNA-target components of RNAi machinery, including AGO-NAUTE1/2 and DICER-LIKE1 (Harmoko et al., 2013; Wang et al., 2021). Interestingly, cotton leaf curl multan geminivirus (CLCuMuV) and tomato yellow leaf curl China virus (TYLCCNV) employ effector protein V2 to repress the activity of CAMTA3 by disrupting its interaction with CaM3 and suppress plant antiviral responses (Wang et al., 2021). This example not only extends our knowledge about the role of CAMTA3 in the plant-pathogen arm races from antibacterial and fungal immunity to antiviral immunity, but also provides a novel insight that Ca^{2+}-triggered defense action could be a direct target of effector proteins deployed by pathogens to compromise its host.

Arabidopsis CAMTA3 is also involved in regulating plant defense against insect herbivores (Laluk et al., 2012; Qiu et al., 2012). *camta3* mutant shows increased vulnerable to herbivore attack, and the CaM-binding property of CAMTA3 is required to establish plant resistance to herbivore attack (Qiu et al., 2012). Furthermore, elevated SA level in *atsr1* mutant was found to negatively regulate both basal- and wound-induced biosynthesis of JA, indicating that Ca^{2+}/CaM-CaMTA3 signalling pathway regulates plant response to herbivore attack by modulating SA-JA crosstalk (Qiu et al., 2012). In addition, *camta3* mutant accumulates less amount of insect repellent aliphatic glucosinolates (GS), and this coincides with altered expression of several genes involved in GS metabolism (Laluk et al., 2012). But it is still unclear whether CAMTA3 directly regulates the expression of these genes.

4.1.3 | Other CaM-binding TFs

Other CaM-targeted TFs also contribute to plant defenses by modulating the expression of defense-related genes. WRKY TFs are one of the key regulators in plant immunity (Wani et al., 2021). WRKY7, a member of WRKY IID subfamily, was identified as a CBP by using protein-protein interaction-based library screening (Park et al., 2005). The region corresponding to CaMBD in WRKY7 is conserved among all WRKY IID members (7 members in *Arabidopsis*), and all the tested WRKY IID members were confirmed to be bound by Ca^{2+}/CaM (Park et al., 2005). Loss-of-function mutant of *Arabidopsis* WRKY7 shows elevated resistance to *Pst* DC3000, while plants overexpressing *WRKY7* shows the opposite phenotype (Kim et al., 2006). Loss-of-function mutant of *WRKY11*, another WRKY IID member in *Arabidopsis*, also exhibits increased resistance to both avirulent and virulent strains of *Pst* DC3000; the increased resistance phenotype in *wrky11* single mutants is exacerbated in *wrky11 wrky17* double mutants, indicating that both of two WRKY IID members play a negative role in regulating basal defense (Journot-Catalino et al., 2006). A comparison of gene expression profiles reveals that WRKY11 and WRKY17 regulate the expression of defense-related genes in a partially redundant manner (Journot-Catalino et al., 2006). Protein microarray and co-immunoprecipitation analysis indicated that WRKY53 interacts with different CaM isoforms in a Ca^{2+}-dependent manner (Popescu et al., 2007). *wrky53* shows enhanced disease susceptibility, while *WRKY53*-overexpression mutants display enhanced resistance to pathogens, suggesting that WRKY53 plays a positive role in plant immunity (Murray et al., 2007). WRKY53 also negatively regulates plant defense against insects by negatively regulating the expression of *lipoxygenase 3 (LOX3)* and *LOX4* which are associated with the biosynthesis of JA (Jiao, Li, et al., 2022). Further studies are required to elucidate the linkage between Ca^{2+}/CaM signalling and the functions of these CaM-binding TFs in plant defenses against pathogens and/or herbivores.

In *Arabidopsis*, TGACG-binding factor 3 (TGA3), a basic leucine zipper TF, was identified to be a CBP; TGA3 binds to C/G-box in the promoter of *CaM3*, and this binding is facilitated by the interaction between TGA3 and CaM (Szymanski et al., 1996). Among the 10 TGAs in *Arabidopsis* genome (Jakoby et al., 2002), seven TGAs (TGA1 to TGA7) interact with NPR1 (Després et al., 2003), which is a SA receptor involved in plant immune response (Ding et al., 2018). Pathogen-induced expression of *PR1* is compromised in *tga3* mutant plants; moreover, TGA3 is required for local defense, but not for SAR (Kesarwani et al., 2007). However, whether and how Ca^{2+}/CaM signalling is involved in the regulation of TGA3 in plant disease resistance remain to be addressed. While screening a cDNA expression library using HRP-labelled CaM2 as a probe, CBNAC, a NAC TF in *Arabidopsis*, was identified to be a CBP, and their interaction was confirmed by yeast split ubiquitin assay and CaM overlay assay (Kim et al., 2007). *cbnac* null mutant shows increased resistance to *Pst* DC3000, while plants overexpressing *CBNAC* exhibits the opposite phenotype in pathogen resistance (Kim et al., 2007). CBNAC is involved in plant defense by regulating the expression of *PR1*. It is speculated that, under normal growth conditions, CBNAC interacts with Suppressor of NPR1-1 Inducible 1 (SNI1) to form SNI1-CBNAC complex and binds to the promoter of *PR1* to suppress its expression. Under pathogen attacks, CaM competitively binds with CBNAC to disrupt SNI1-CBNAC complex and thus removes the inhibition of *PR1* expression (Kim et al., 2012).

4.2 | Transcriptional regulation via other CaM-binding proteins

In addition to CaM-binding TFs, other kinds of CBPs also play a role in transcriptional regulation in plant responses to biotic stresses. MAPK cascade activates defense signalling through three-stage phosphorylation, thereby activating various TFs to coordinate the transcriptional reprogramming of defense genes (Meng & Zhang, 2013). MAPK phosphatase (MKP) is a negative regulator of MAPK-mediated signal transduction. The linkage between MKP and Ca^{2+}/CaM signalling was first uncovered in tobacco, where NtMKP1 was identified as a CBP (Yamakawa et al., 2004). NtMKP1 negatively regulates wounding response, resistance against necrotrophic pathogen *B. cinerea*, and lepidopteran herbivores, *Mamestra brassicae* and

Spodoptera litura (Yamakawa et al., 2004). NtMKP1 homologues in rice and *Arabidopsis* have also been identified to be CBPs, and AtMKP1 in *Arabidopsis* contains two CaMBDs (Katou et al., 2007; Lee et al., 2008). The phosphatase activity of AtMKP1 is positively regulated by CaM in a Ca^{2+}-dependent manner (Lee et al., 2008). *Arabidopsis mkp1* null mutant exhibits stunned growth and constitutive activation of defense responses, including elevated accumulation of camalexin and SA, and enhanced resistance to *Pst* DC3000 (Bartels et al., 2009). Further evidence indicates that MPK6, but not MPK3, is required for the constitutive defense phenotype exhibited in *mkp1*, suggesting a negative regulation of MKP1 on MPK6-mediated PTI (Anderson et al., 2011). In rice, *OsMKP1* is significantly induced by wounding, and overexpression of *OsMKP1* results in reduced wound-induced MAPK cascades, which provides additional evidence for MKP1 acting as negative regulator in MAPK signalling pathway (Katou et al., 2007). Interestingly, MKP1 is feedback regulated by MAPK cascade; AtMKP1 is phosphorylated by MPK6, which improves the stability of AtMKP1 (Jiang et al., 2017).

IQ67-domain 1 (IQD1) belongs to a family that comprises 33 genes in *Arabidopsis*, all encoding proteins containing a plant-specific domain of 67 conserved amino acids termed the IQ67 domain. It has been suggested that IQD1 may provide scaffolds to facilitate cellular transport of RNA-protein complexes along microtubules via kinesin motor proteins, and thus could control gene expression and protein sorting (Bürstenbinder et al., 2013). IQD1 has been identified to be a CBP that is located in the nucleus, and functions as a positive regulator of GS accumulation and plant defense against herbivore attacks (Levy et al., 2005). The expression of *IQD1* is induced by mechanical wounding, and IQD1 may serve as a signalling component to integrate intracellular Ca^{2+} signal to GS biosynthesis in response to insect attacks (Levy et al., 2005). IQD1 is also involved in plant resistance to the necrotrophic fungus *B. cinerea* (Barda & Levy, 2022). However, the underlying molecular mechanisms and the linkage to Ca^{2+}/CaM signal of how IQD1 is involved in plant defense responses await further investigations. Besides IQD1, IQD14 has also been identified to be bound by CaM, CML13 and CML14 in vitro in the presence or absence of Ca^{2+} (Teresinski et al., 2023). In addition, based on the presence of tandem IQ domains nearby the CaMBD, CAMTAs have also been identified to be bound by CaM/CMLs, such as CML13 and CML14 (Teresinski et al., 2023). Interestingly, the mutation in IQ domain of CAMTA3 caused a gain-of-function phenotype, while one amino acid mutation in CaMBD of CAMTA3 resulted in a loss-of-function phenotype in plant immunity (Yuan et al., 2021).

5 | CA^{2+} TRANSIENTS IN RESPONSE TO BIOTIC STRESSES ARE MODULATED BY CAMS/CMLS

While intracellular Ca^{2+} transients induced by pathogen attacks serve as essential cell signals to trigger defense responses, these Ca^{2+} transients could be regulated by Ca^{2+}/CaM signalling through feedback loops involving Ca^{2+} permeable channels, Ca^{2+} pumps and Ca^{2+} antiporters (Figure 4).

Cyclic nucleotide gated channels (CNGCs) are Ca^{2+} permeable cation channels that mainly localized on the plasma membrane (PM) and are regulated by CaM and cyclic nucleotides, such as cNMPs (Jarratt-Barnham et al., 2021). The C-terminal of CNGC contains a cyclic nucleotide binding domain (CNBD) overlapping with a CaMBD, and an IQ motif (Jarratt-Barnham et al., 2021). Notably, the N-terminal of *Arabidopsis* CNGC12 contains a CaMBD (DeFalco, Marshall, et al., 2016). All CNGCs in *Arabidopsis* interact with CaMs via CaMBD domains or IQ motifs (Fischer et al., 2017). It was speculated that CaM functions as a Ca^{2+}-sensing subunit in the channel complex and mediates subunit assembly (DeFalco, Marshall, et al., 2016; DeFalco, Moeder, et al., 2016). *cngc2* null mutant, also known as *defense no death 1* (*dnd1*), as its name implies, was isolated by its failure to trigger HR induced by avirulent bacterial pathogens; *dnd1* exhibits an autoimmune phenotype with enhanced basal resistance to pathogens (Clough et al., 2000). By electrophysiological experiments, it was found that a cAMP-induced Ca^{2+} current is compromised in *cngc2* mutant, and this Ca^{2+} current is associated with NO production (Ali et al., 2007). *Arabidopsis* null mutant of *CNGC4* (namely *dnd2*) also shows spontaneous lesions and reduced ETI-mediated HR as well as increased basal resistance to pathogens (Balague et al., 2003). A recent study shows that when Ca^{2+} supply is sufficient in plants, CNGC2 and CNGC4 can be assembled into a functional Ca^{2+} channel that is blocked by CaM7 in the resting state (Tian et al., 2019). Under pathogen attack, BIK1, as a member of RLCK, activates Ca^{2+} channels through phosphorylation of CNGC4, thereby causing an increase in cytosolic Ca^{2+} concentration; these results suggest that CNGC-mediated Ca^{2+} influx is an intermediate bridge for PAMP to trigger PTI (Tian et al., 2019). Mutations of CNGC11 and CNGC12 also result in abnormal defense responses in *Arabidopsis*; single knockout mutants of CNGC11 or CNGC12, and a double RNAi knockdown mutant *cngc11cngc12* show significantly decreased resistance to avirulent pathogens (Yoshioka et al., 2006). *Constitutive expresser of PR genes22* (*cpr22*) is a gain-of-function mutant that causes constitutive activation of defense responses, such as spontaneous formation of lesions and HR-like responses in *Arabidopsis* (Yoshioka et al., 2006). *cpr22* mutant carries a 3 kb deletion, which results in the mutation in both CNGC11 and CNGC12, forming a chimeric channel CNGC11/12 (Yoshioka et al., 2006). Subsequent studies indicate that spontaneous lesion in *cpr22* is caused by the aberrant regulation of programmed cell death (PCD), and the chimeric channel CNGC11/12 mediates Ca^{2+} influx that is necessary for HR development (Urquhart et al., 2007). CNGC19 and CNGC20 function as Ca^{2+} channels by forming possible homo- and heteromeric protein complexes (Yu et al., 2019). BAK1 and SERK4, as coreceptors of FLS2, BRI1 and some other LRR-RLKs, can directly phosphorylate CNGC20 and regulate the stability of CNGC20. In *bak1/serk4* double mutant, CNGC20 is maintained at a nonphosphorylated state, which increases its stability and results in excessive Ca^{2+} influx into cells, leading to the induction of cell death (Yu et al., 2019). *cngc20-4*, a missense mutant of *CNGC20* identified

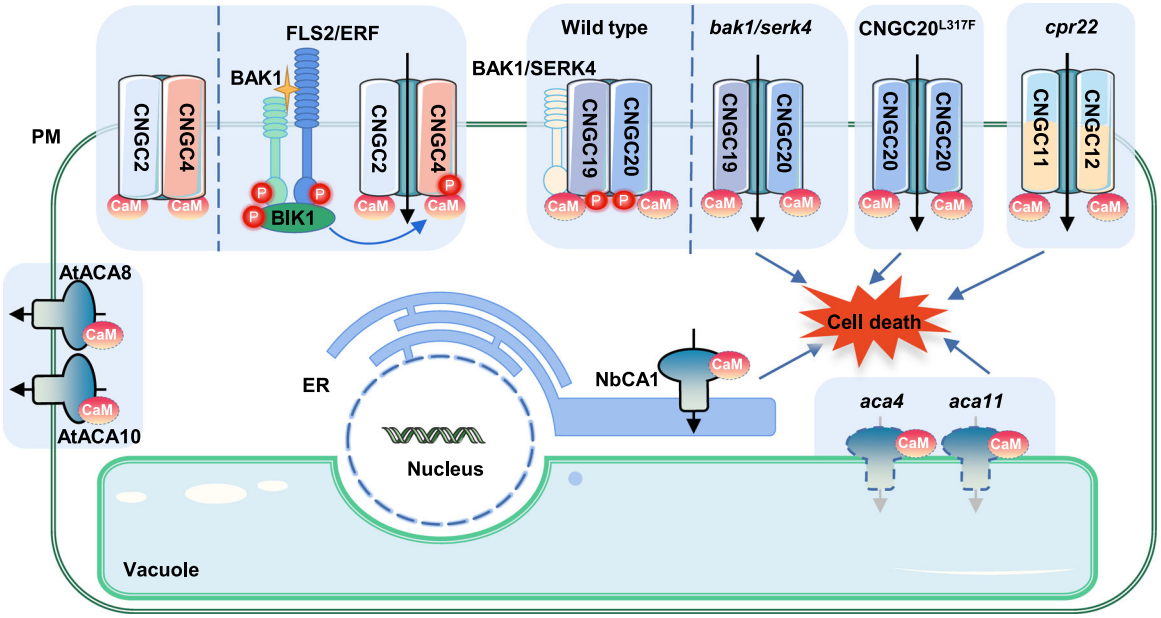

FIGURE 4 Involvement of CaMs/CMLs in regulating CNGC-mediated Ca^{2+} influx and ACA-mediated Ca^{2+} efflux in plant immunity. ACAs and CNGCs are targeted by CaMs/CMLs. CNGC forms homologous or heterogeneous isomers at PM to mediate Ca^{2+} influx. In the absence of pathogens, CNGC2 and CNGC4 homotetramers are inactive, whereas heterotetramers allow proper cation flux into the cytoplasm. Under pathogen infections, BIK1 is activated and phosphorylates CNGC4 to release CaM-mediated inhibition and induce Ca^{2+} influx. Excessive influx of Ca^{2+} via CNGCs induces cell death through an unclear mechanism. BAK1 negatively regulates Ca^{2+} transients through the phosphorylation of CNCG20 to facilitate its degradation. CNGC19 and CNGC20 form protein complexes at the plasma membrane (PM). The *crp22* mutant expressed chimeric CNGC11/12 exhibits programmed cell death (PCD). In the gain-of function mutant, the accumulation of CNGC19/CNGC20 channels leads to the influx of Ca^{2+}, and ultimately leads to cell death. ACAs help establishing a low cytoplasmic Ca^{2+} concentration by exporting Ca^{2+} to the apoplast and/or the vacuole. Mutations of ACA8 and ACA10, both located in the PM, decrease the generation of Ca^{2+} signal that is induced by flg22 and reduce pathogen resistance, but mutations of ACA4 and ACA11, both located in the tonoplast, result in constitutive defense responses. Endoplasmic reticulum (ER)-located Ca^{2+}-ATPases, such as NbCA1, also regulate intracellular Ca^{2+} oscillations and play an important role in plant defense. [Color figure can be viewed at wileyonlinelibrary.com]

in the background of *eds1* mutant, shows a typical self-activating immune phenotype, such as plant dwarfism, constitutive disease resistance gene expression, excessive accumulation of SA, and enhanced PTI and ETI responses (Zhao et al., 2021), suggesting that CNGC20 mediates plant immune response in an EDS1-independent manner. In addition, CNGC19 was found to be involved in plant defense against herbivorous insects (Meena et al., 2019). Further studies are required to clarify the potential role of Ca^{2+}/CaM signalling in CNGC-regulated herbivore resistance.

Similar to CNGCs, Mildew resistance Locus O (MLO) has also been found to act as a Ca^{2+} channel to regulate Ca^{2+} transients by using single cell Ca^{2+} imaging and patch clamp technologies (Gao et al., 2022). *MLO* was first identified to be a plant specific recessive disease resistance gene in barley (*Hordeum vulgare*); homozygous recessive *mlo* mutants confer broad spectrum disease resistance to the biotrophic powdery mildew fungus, *Blumeria graminis* (Jorgensen, 1992). However, powdery mildew-resistant *mlo* plants exhibit enhanced susceptibility to other fungal pathogens, such as *Magnaporthe grisea* (Jarosch et al., 1999) and *Bipolaris sorokiniana* (Kumar et al., 2001), suggesting that MLO plays different roles in resistances to diverse fungal pathogens. *MLO* family genes have been isolated from *Arabidopsis*, wheat, rice and other plants (Acevedo-Garcia et al., 2014; Chen et al., 2006; Konishi et al., 2010; Liu & Zhu, 2008). Barley *MLO* encodes a PM-localized protein containing seven transmembrane domains and shares certain degree of homology with G-protein coupled receptor proteins in animals (Panstruga, 2005b). MLO has been identified to be a CBP, and CaM binding is required for the normal function of MLO to repress plant defense against pathogen infections in barley (Kim, Panstruga, et al., 2002). Rice MLO was also identified to be a CBP in the searching of CBPs responsive to pathogen infection (Kim, Lee, et al., 2002). Notably, the CaMBD of MLO homologues are conserved in higher plants (Kim, Lee, et al., 2002). CaMLO2 from pepper is also a CBP that negatively regulates plant immunity; silencing of *CaMLO2* in pepper results in enhanced resistance against virulent *Xanthomonas campestris*, which is associated with impaired susceptibility cell-death response and reduced bacterial growth, as well as accelerated bursts of ROS (Kim & Hwang, 2012). CaMLO2 specifically interacts with CaCaM1 and translocates cytoplasmic CaCaM1 to the PM, leading to the suppression of *Xanthomonas* effector-triggered Ca^{2+} influx, hypersensitive cell death and defense responses (Kim et al., 2014). Ectopic expression of *CaMLO2* in *Arabidopsis* leads to enhanced susceptibility to biotrophic oomycete *Hyaloperonospora arabidopsidis* and hemibiotrophic pathogen *Pst* DC3000 (Kim & Hwang, 2012). MLO2, MLO6, and MLO12 in

Arabidopsis are considered as co-orthologs of barley MLO (Panstruga, 2005a). *Arabidopsis mlo2* single mutant shows incomplete resistance to two powdery mildew species, *Golovinomyces orontii* and *Golovinomyces cichoracearum*, but the triple mutant *mlo2 mlo6 mlo12* has full resistance to pathogens (Consonni et al., 2006). Penetration 3 (PEN3), also known as Pleiotropic Drug Resistance 8 (PDR8) or ATP Binding Cassette (ABC) Transporter G Family Member 36 (ABCG36), is required for the nonhost resistance of *mlo* mutants in *Arabidopsis* (Consonni et al., 2006). Notably, *pen3* knockout mutant shows impaired resistance to nonhost pathogens (Stein et al., 2006). Studies show that PEN3 interacts with CaM, and the interaction is necessary for nonhost resistance in *Arabidopsis* (Campe et al., 2016). It is reasonable to speculate that MLO-mediated pathogen resistance and nonhost immunity interplay and this process involves Ca^{2+}/CaM signalling. However, it is still unclear whether and how Ca^{2+}/CaM regulates the Ca^{2+} channel function of MLO proteins.

In addition to Ca^{2+} permeable channels, Ca^{2+}/CaM also regulates Ca^{2+} pumps and Ca^{2+} antiporters to mediate Ca^{2+} efflux during plant-microbe interactions. Autoinhibited Ca^{2+}-ATPase (ACA), which contains a CaM binding domain and a self-inhibition domain at its N-terminal, belongs to a subgroup of P-type ATPases (Astegno et al., 2017; Boursiac et al., 2010; Zhu et al., 2010). Double knockout mutants of two tonoplast-localized Ca^{2+} pumps in *Arabidopsis*, ACA4 and ACA11, display a high frequency of HR-like lesions, and further genetic evidence suggested that vacuolar-localized Ca^{2+} pumps regulated SA-mediated PCD (Boursiac et al., 2010). Other studies have shown that the disruption of PM-localized ACA8 or ACA10 leads to changes in plant immunity (Yang et al., 2017). Notably, CML36 can also interact with ACA8, and the interaction activates the pump activity of ACA8 (Astegno et al., 2017). NbCA1, an ER-localized Ca^{2+} pump in tobacco, was also found to mediate Ca^{2+} efflux and regulate PCD during plant innate immune response (Zhu et al., 2010). However, how CaM regulates Ca^{2+} permeable channels, Ca^{2+} pumps and antiporters, and how Ca^{2+} transients are regulated by CaM-mediated signalling in plants are far from clear and deserve further investigations.

6 | CAM/CML-MEDIATED REGULATION OF ROS HOMEOSTASIS

Increase in intracellular Ca^{2+} and the outburst of ROS are common early cellular events in plant responses to pathogen infection or elicitor treatment (Apostol et al., 1989; Atkinson et al., 1996; Lecourieux et al., 2002; Marcec et al., 2019). There is an extensive interplay between Ca^{2+} and ROS to amplify the immune signalling pathway (Gilroy et al., 2016; Marcec et al., 2019). It has been well known that exogenous application of H_2O_2 triggers increases in cytosolic Ca^{2+} by activating the Ca^{2+}-permeable channels (Rentel & Knight, 2004; Wu et al., 2020). On the other hand, ROS signals during immune responses are generated mainly from NADPH oxidases of the respiratory burst oxidase homologues (RBOHs) family located on the membrane, and are well known to be regulated by Ca^{2+} signal (Waszczak et al., 2018). *Arabidopsis* RBOHs contain NADPH binding domain, FAD binding domain and six conserved transmembrane domains, as well as phosphorylation sites and two conserved Ca^{2+} binding EF-hand in the N-terminal (Keller et al., 1998) (Figure 5a). H_2O_2 production during oxidative burst is dependent on continuous Ca^{2+} influx, which activates RBOH directly via its cytoplasmic Ca^{2+}-binding EF-hand motifs (Ogasawara et al., 2008; Xing et al., 1997). Ca^{2+} signal also activates RBOHs through protein phosphorylation mediated by Ca^{2+}-dependent kinases like CDPKs and Ca^{2+}/CaM-dependent protein kinases (CCaMKs). In rice, the CCaMK, also known as doesn't make infections 3 (OsDMI3), phosphorylates OsRbohD at Ser-191 to activate its NADPH oxidase activity for ROS generation (Wang, Shen, et al., 2023). Knockout of RbohD or application of inhibitors to eliminate ROS accumulation results in decreased Ca^{2+} spiking; meanwhile, stress-induced ROS production can be severely attenuated by treatment with Ca^{2+} channel blockers (Ranf et al., 2011). BIK1 can activate Ca^{2+} channels and phosphorylate PM-localized RbohD when plants are attacked by pathogens (Kadota et al., 2014; Li et al., 2014). *Arabidopsis* RbohD is involved in the production of ROS in response to pathogen-derived elicitors (Lee et al., 2020; Nühse et al., 2007). NAD kinases (NADKs), which supply NADP cofactor for ROS production through NADPH oxidases, have been identified to be CBPs (Harding, Oh, & Roberts, 1997; Karita et al., 2004; Turner et al., 2004). Recently, the exact NADK protein responsible for Ca^{2+}/CaM-dependent NAD^+ kinase activity has been identified and named NADK-CaM dependent (NADKc) in *Arabidopsis*; NADKc containing a CaMBD in its N-terminal region, is located at the mitochondrial periphery and functions in sustaining pathogen-derived elicitor-induced ROS outbreak (Dell'Aglio et al., 2019). Together, these discoveries support that cytosolic Ca^{2+} alone, and/or together with CaM, could stimulate the production of ROS.

Since excessive accumulation of ROS can cause damage to plant cells, plants have evolved ROS scavenging system to maintain ROS homeostasis. Ca^{2+}/CaMs play important roles in ROS homeostasis regulation by targeting ROS scavenging enzymes. Catalases, which catalyze the hydrolysis of hydrogen peroxide and play an important role in controlling ROS homeostasis, have three members, that is, CAT1, CAT2, and CAT3, in *Arabidopsis* (Du et al., 2008). *Arabidopsis* CAT2 is inhibited by SA and plays a role in balancing plant immune responses mediated by SA and JA (Yuan et al., 2017). *Arabidopsis* CAT3 has been verified to bind CaM in a Ca^{2+}-dependent manner, and a stretch of amino acid (425 to 451) is the CaMBD recognized by Ca^{2+}/CaM (Yang & Poovaiah, 2002b). The regions corresponding to CaMBD of CAT3 are conserved in CAT homologues from plants but not from animals or bacteria, suggesting that CATs from other plants may also be regulated by Ca^{2+}/CaM. Indeed, the purified CAT from tobacco binds to Ca^{2+}/CaM and the activity is significantly stimulated by Ca^{2+}/CaM (Yang & Poovaiah, 2002b). *Arabidopsis* MPK8 is also a CBP, and its activation by mechanical wounding requires both CaM binding and upstream MAPKK-mediated phosphorylation. Activated MPK8 suppresses ROS accumulation via transcriptional regulation of *RbohD*. Thus, Ca^{2+}/CaM-mediated regulation and MAPK cascade coordinately regulate MPK8 to fine-tune ROS homeostasis under

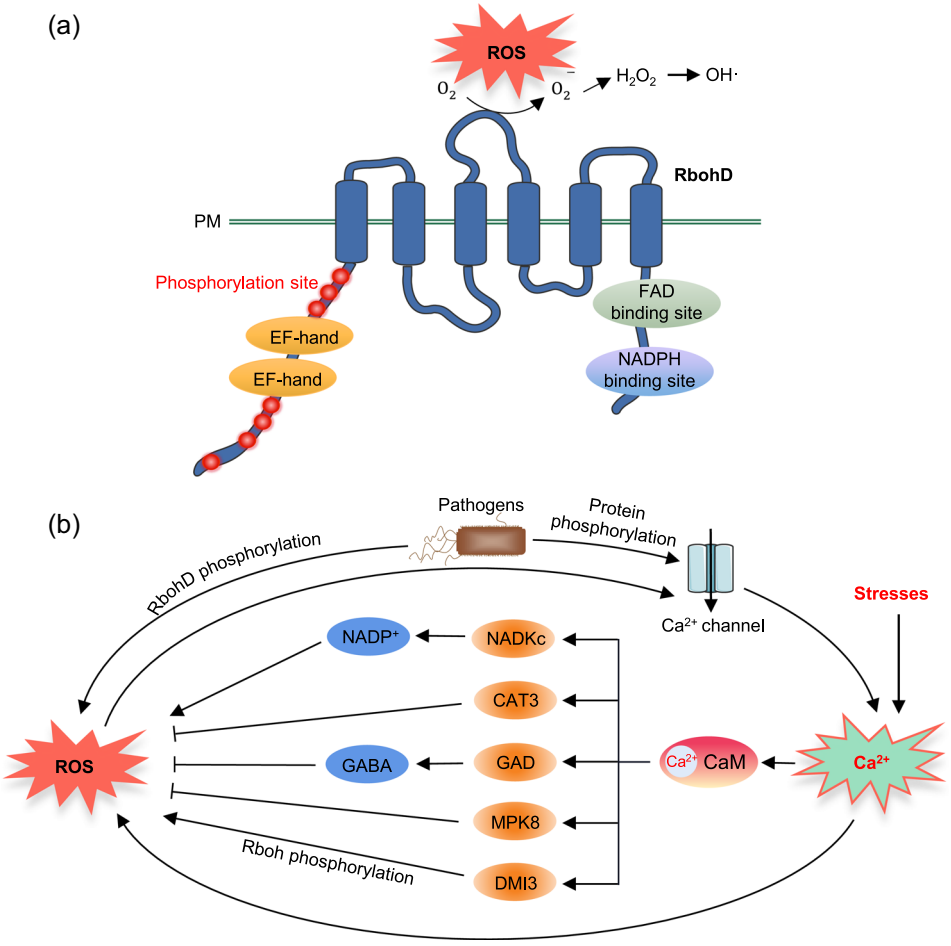

FIGURE 5 CaM/CML-mediated regulation of ROS homeostasis in responses to biotic stresses. (a) Protein structure and functional domains of RbohD in plants. (b) CaM/CML-mediated regulation of ROS homeostasis. Under pathogen invasions, Ca^{2+} channels and RbohD are phosphorylated and activated, leading to the production of cytoplasmic Ca^{2+} transients and ROS accumulation. ROS can further activate Ca^{2+} channels. Ca^{2+} signals activate RbohD by binding with the N-terminal EF-hand motifs, and mediate the phosphorylation of RbohD through Ca^{2+}-regulated protein kinases to initiate ROS production. Ca^{2+}/CaM signalling regulates ROS homeostasis by targeting NADKc. NADKc phosphorylates NAD^+ to generate $NADP^+$ which is further converted to NADPH, the substrate of RBOH proteins. CCaMK (also named DMI3) is also targeted by Ca^{2+}/CaM signalling and positively regulates ROS production by mediating phosphorylation of RbohB. One the other hand, Ca^{2+}/CaM signalling targets CAT to scavenge the accumulated ROS. MPK8, which is induced by damages and negatively regulates ROS production, is also regulated by Ca^{2+}/CaM signalling. In addition, the production of GABA, which is regulated by Ca^{2+}/CaM-targeted GAD under herbivore attacks, is associated with ROS homeostasis. [Color figure can be viewed at wileyonlinelibrary.com]

wounding stress (Takahashi et al., 2011) (Figure 5b). But whether MPK8 is involved in pathogen and/or herbivore resistance remains to be investigated.

Gamma-aminobutyrate (GABA) is a ubiquitous four-carbon non-protein amino acid that participates in the defense response of plants to pathogens and herbivores (Tarkowski et al., 2020). GABA shunt inhibits the accumulation of ROS and reduces ROS-mediated cell death (MacGregor et al., 2003) (Figure 5b). Glutamate decarboxylase (GAD), as a key enzyme, catalyzes the production of GABA and is characterized as a CBP (Gut et al., 2009; Snedden et al., 1996). The constitutive overexpression of *GAD* in tobacco improves GABA levels and confers resistance to northern root-knot nematode (McLean et al., 2003) and tobacco budworm larvae (MacGregor et al., 2003) possibly through a GABA-based mechanism. It has also been suggested that the interaction between CML8 and GAD4 promotes GABA biosynthesis, which further increases the expression of defense-related genes, and thereby improves resistance to herbivore *Plutella xylostella* in *Arabidopsis* (Jiao, Guo, et al., 2022).

7 | CONCLUDING REMARKS AND PERSPECTIVES

Ca^{2+} represents a universal second messenger playing a crucial role in plant responses to environmental stimuli. Ca^{2+} signalling can be divided into three steps, that is, the generation of Ca^{2+} signal, the decoding of Ca^{2+} signal, and the relaying of Ca^{2+} signal to specific adaptive responses. CaMs and CMLs belong to a major class of Ca^{2+}

sensors that decode cryptic Ca^{2+} signal. Although much progress has been made on roles of CaMs/CMLs and CBPs in plant responses to biotic stresses (Table 1), many efforts are still needed to expand our knowledge about downstream target proteins of CaMs/CMLs and their physiological, biochemical and molecular functions, as well as molecular mechanisms by which they mediate plant responses to biotic stresses.

Many questions are still required to be addressed in the future. For example, how to distinguish different Ca^{2+} oscillation produced by various stresses, and how specific Ca^{2+} signals are precisely decoded by CaMs/CMLs under biotic stresses are largely unclear. The generation of Ca^{2+} transients in plant defenses against pathogens and herbivores depends on the coordination of diverse Ca^{2+} conducting proteins including CaM-binding Ca^{2+} permeable channels and Ca^{2+} pumps, such as CNGCs, MLOs and ACAs. But how multiple Ca^{2+} channels/pumps from diverse gene families coordinate and contribute to the generation of specific Ca^{2+} signature during PTI and ETI, and the underlying molecular mechanisms of how CaMs/CMLs regulate the activities of Ca^{2+} channels/pumps are still unclear. Plant CaMs/CMLs have multiple isoforms, but lack specific enzymatic/biochemical functions. CaMs/CMLs usually bind to and regulate the activities of a wide range of downstream CBPs, but the specificity, preference and flexibility of the interaction between diverse CaM/CML isoforms and their CBPs still need to be clarified. Although some progress has been made in the role of CMLs in biotic stress responses, their target proteins are largely unidentified. Considering the large amount of CML members in plant genome, further studies are necessary to identify their target proteins and characterize their functional significance in biotic stress responses. New techniques based on high through-put analyses, multiomics, and technologies with high optical resolution will help us address these issues. Advances in molecular mechanisms in CaM/CML-mediated regulation in plant biotic stress responses will advance our understanding of the most striking characteristics, versatility, efficiency, and accuracy of Ca^{2+} signalling pathways.

In addition, most of these progresses have been made in model plant *Arabidopsis*, the roles of CaMs/CMLs and CBPs in biotic stress responses in crops are still largely unclear. With the technical advances in genetic transformation and genome editing, more research should be focused on crops. By modulating the expression of CaMs/CMLs and/or CBPs or the affinity of their interactions through diverse strategies, such as gene engineering using stress-specific responsive promoters, CRISPR/Cas-mediated genome editing of cis-regulatory elements, CRISPR/Cas-derived gene activation and prime editing, crops with more tolerance to biotic stresses and without growth impairment could be foreseeably developed, and this will significantly facilitate the sustainability of our agricultural industry and food security.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

This is a review article. Do not have data needed to share.

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REFERENCES

Abbas, N., Maurya, J.P., Senapati, D., Gangappa, S.N. & Chattopadhyay, S. (2014) Arabidopsis CAM7 and HY5 physically interact and directly bind to the HY5 promoter to regulate its expression and thereby promote photomorphogenesis. *The Plant Cell*, 26(3), 1036–1052. Available from: https://doi.org/10.1105/tpc.113.122515

Acevedo-Garcia, J., Kusch, S. & Panstruga, R. (2014) Magical mystery tour: MLO proteins in plant immunity and beyond. *New Phytologist*, 204(2), 273–281. Available from: https://doi.org/10.1111/nph.12889

Ali, G.S., Reddy, V.S., Lindgren, P.B., Jakobek, J.L. & Reddy, A.S.N. (2003) Differential expression of genes encoding calmodulin-binding proteins in response to bacterial pathogens and inducers of defense responses. *Plant Molecular Biology*, 51(6), 803–815.

Ali, R., Ma, W., Lemtiri-Chlieh, F., Tsaltas, D., Leng, Q., von Bodman, S. et al. (2007) Death don't have no mercy and neither does calcium: Arabidopsis CYCLIC NUCLEOTIDE GATED CHANNEL2 and innate immunity. *The Plant Cell*, 19(3), 1081–1095.

Anderson, J.C., Bartels, S., Besteiro, M.A.G., Shahollari, B., Ulm, R. & Peck, S.C. (2011) Arabidopsis MAP Kinase Phosphatase 1 (AtMKP1) negatively regulates MPK6-mediated PAMP responses and resistance against bacteria: MKP1 negatively regulates PAMP responses. *The Plant Journal*, 67(2), 258–268. Available from: https://doi.org/10.1111/j.1365-313X.2011.04588.x

Apostol, I., Heinstein, P.F. & Low, P.S. (1989) Rapid stimulation of an oxidative burst during elicitation of cultured plant cells: role in defense and signal transduction. *Plant Physiology*, 90(1), 109–116. Available from: https://doi.org/10.1104/pp.90.1.109

Astegno, A., Bonza, M.C., Vallone, R., La Verde, V., D'Onofrio, M., Luoni, L. et al. (2017) Arabidopsis calmodulin-like protein CML36 is a calcium (Ca(2+)) sensor that interacts with the plasma membrane Ca(2+)-ATPase isoform ACA8 and stimulates its activity. *Journal of Biological Chemistry*, 292(36), 15049–15061. Available from: https://doi.org/10.1074/jbc.M117.787796

Atkinson, M.M., Midland, S.L., Sims, J.J. & Keen, N.T. (1996) Syringolide 1 triggers Ca2+ influx, K+ efflux, and extracellular alkalization in soybean cells carrying the disease-resistance gene Rpg4. *Plant Physiology*, 112(1), 297–302. Available from: https://doi.org/10.1104/pp.112.1.297

Bähler, M. & Rhoads, A. (2002) Calmodulin signaling via the IQ motif. *FEBS Letters*, 513(1), 107–113. Available from: https://doi.org/10.1016/s0014-5793(01)03239-2

Balague, C., Lin, B., Alcon, C., Flottes, G., Malmstrom, S., Kohler, C. et al. (2003) HLM1, an essential signaling component in the hypersensitive response, is a member of the cyclic nucleotide-gated channel ion channel family. *Plant Cell*, 15(2), 365–379.

Barda, O. & Levy, M. (2022) IQD1 involvement in hormonal signaling and general defense responses against botrytis cinerea. *Frontiers in Plant*

Science, 13, 845140. Available from: https://doi.org/10.3389/fpls.2022.845140

Bartels, S., Anderson, J.C., González Besteiro, M.A., Carreri, A., Hirt, H., Buchala, A. et al. (2009) MAP kinase phosphatase1 and protein tyrosine phosphatase1 are repressors of salicylic acid synthesis and SNC1-mediated responses in Arabidopsis. *The Plant Cell*, 21(9), 2884–2897.

Bi, G., Su, M., Li, N., Liang, Y., Dang, S., Xu, J. et al. (2021) The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. *Cell*, 184(13), 3528–3541. Available from: https://doi.org/10.1016/j.cell.2021.05.003

Boonburapong, B. & Buaboocha, T. (2007) Genome-wide identification and analyses of the rice calmodulin and related potential calcium sensor proteins. *BMC Plant Biology*, 7, 4. Available from: https://doi.org/10.1186/1471-2229-7-4

Bouché, N., Yellin, A., Snedden, W.A. & Fromm, H. (2005) Plant-specific calmodulin-binding proteins. *Annual Review of Plant Biology*, 56, 435–466. Available from: https://doi.org/10.1146/annurev.arplant.56.032604.144224

Boursiac, Y., Lee, S.M., Romanowsky, S., Blank, R., Sladek, C., Chung, W.S. et al. (2010) Disruption of the vacuolar calcium-ATPases in Arabidopsis results in the activation of a salicylic acid-dependent programmed cell death pathway. *Plant Physiology*, 154(3), 1158–1171. Available from: https://doi.org/10.1104/pp.110.159038

Bürstenbinder, K., Savchenko, T., Müller, J., Adamson, A.W., Stamm, G., Kwong, R. et al. (2013) Arabidopsis calmodulin-binding protein IQ67-domain 1 localizes to microtubules and interacts with kinesin light chain-related protein-1. *Journal of Biological Chemistry*, 288(3), 1871–1882. Available from: https://doi.org/10.1074/jbc.M112.396200

Campe, R., Langenbach, C., Leissing, F., Popescu, G.V., Popescu, S.C., Goellner, K. et al. (2016) ABC transporter PEN3/PDR8/ABCG36 interacts with calmodulin that, like PEN3, is required for Arabidopsis nonhost resistance. *New Phytologist*, 209(1), 294–306. Available from: https://doi.org/10.1111/nph.13582

Chen, Z., Hartmann, H.A., Wu, M.J., Friedman, E.J., Chen, J.G., Pulley, M. et al. (2006) Expression analysis of the AtMLO gene family encoding plant-specific seven-transmembrane domain proteins. *Plant Molecular Biology*, 60(4), 583–597. Available from: https://doi.org/10.1007/s11103-005-5082-x

Cheng, H.Q., Han, L.B., Yang, C.L., Wu, X.M., Zhong, N.Q., Wu, J.H. et al. (2016) The cotton MYB108 forms a positive feedback regulation loop with CML11 and participates in the defense response against Verticillium dahliae infection. *Journal of Experimental Botany*, 67(6), 1935–1950. Available from: https://doi.org/10.1093/jxb/erw016

Chiasson, D., Ekengren, S.K., Martin, G.B., Dobney, S.L. & Snedden, W.A. (2005) Calmodulin-like proteins from Arabidopsis and tomato are involved in host defense against Pseudomonas syringae pv. tomato. *Plant Molecular Biology*, 58(6), 887–897. Available from: https://doi.org/10.1007/s11103-005-8395-x

Choi, H.W., Lee, D.H. & Hwang, B.K. (2009) The pepper calmodulin gene CaCaM1 is involved in reactive oxygen species and nitric oxide generation required for cell death and the defense response. *Molecular Plant-Microbe Interactions®*, 22(11), 1389–1400. Available from: https://doi.org/10.1094/MPMI-22-11-1389

Choi, M.S., Kim, M.C., Yoo, J.H., Moon, B.C., Koo, S.C., Park, B.O. et al. (2005)). Isolation of a calmodulin-binding transcription factor from rice (*Oryza sativa* L.). *Journal of Biological Chemistry*, 280(49), 40820–40831. Available from: https://doi.org/10.1074/jbc.M504616200

Clough, S.J., Fengler, K.A., Yu, I., Lippok, B., Smith, R.K. & Bent, A.F. (2000) The Arabidopsis dnd1 "defense, no death" gene encodes a mutated cyclic nucleotide-gated ion channel. *Proceedings of the National Academy of Sciences of the United States of America*, 97(16), 9323–9328. Available from: https://doi.org/10.1073/pnas.150005697

Consonni, C., Humphry, M.E., Hartmann, H.A., Livaja, M., Durner, J., Westphal, L. et al. (2006) Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nature Genetics*, 38(6), 716–720. Available from: https://doi.org/10.1038/ng1806

Cook, D.E., Mesarich, C.H. & Thomma, B.P.H.J. (2015) Understanding plant immunity as a surveillance system to detect invasion. *Annual Review of Phytopathology*, 53, 541–563. Available from: https://doi.org/10.1146/annurev-phyto-080614-120114

DeFalco, T.A., Bender, K.W. & Snedden, W.A. (2009) Breaking the code: Ca2+ sensors in plant signalling. *Biochemical Journal*, 425(1), 27–40. Available from: https://doi.org/10.1042/BJ20091147

DeFalco, T.A., Marshall, C.B., Munro, K., Kang, H.G., Moeder, W. & Ikura, M. et al. (2016) Multiple calmodulin-binding sites positively and negatively regulate Arabidopsis CYCLIC NUCLEOTIDE-GATED CHANNEL12. *The Plant Cell*, 28(7), 1738–1751. Available from: https://doi.org/10.1105/tpc.15.00870

DeFalco, T.A., Moeder, W. & Yoshioka, K. (2016) Opening the gates: insights into cyclic nucleotide-gated channel-mediated signaling. *Trends in Plant Science*, 21(11), 903–906. Available from: https://doi.org/10.1016/j.tplants.2016.08.011

Dell'Aglio, E., Giustini, C., Kraut, A., Couté, Y., Costa, A. & Decros, G. et al. (2019) Identification of the Arabidopsis calmodulin-dependent NAD+ kinase that sustains the elicitor-induced oxidative burst. *Plant Physiology*, 181(4), 1449–1458. Available from: https://doi.org/10.1104/pp.19.00912

Després, C., Chubak, C., Rochon, A., Clark, R., Bethune, T., Desveaux, D. et al. (2003) The Arabidopsis NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. *The Plant Cell*, 15(9), 2181–2191. Available from: https://doi.org/10.1105/tpc.012849

Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Y. & Li, X. et al. (2018) Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. *Cell*, 173(6), 1454–1467. Available from: https://doi.org/10.1016/j.cell.2018.03.044

Dobney, S., Chiasson, D., Lam, P., Smith, S.P. & Snedden, W.A. (2009) The calmodulin-related calcium sensor CML42 plays a role in trichome branching. *Journal of Biological Chemistry*, 284(46), 31647–31657. Available from: https://doi.org/10.1074/jbc.M109.056770

Dongus, J.A. & Parker, J.E. (2021) EDS1 signalling: at the nexus of intracellular and surface receptor immunity. *Current Opinion in Plant Biology*, 62, 102039. Available from: https://doi.org/10.1016/j.pbi.2021.102039

Du, L., Ali, G.S., Simons, K.A., Hou, J., Yang, T., Reddy, A.S.N. et al. (2009) Ca2+/calmodulin regulates salicylic-acid-mediated plant immunity. *Nature*, 457(7233), 1154–1158. Available from: https://doi.org/10.1038/nature07612

Du, Y.Y., Wang, P.C., Chen, J. & Song, C.P. (2008) Comprehensive functional analysis of the catalase gene family in *Arabidopsis thaliana*. *Journal of Integrative Plant Biology*, 50(10), 1318–1326. Available from: https://doi.org/10.1111/j.1744-7909.2008.00741.x

Feehan, J.M., Castel, B., Bentham, A.R. & Jones, J.D. (2020) Plant NLRs get by with a little help from their friends. *Current Opinion in Plant Biology*, 56, 99–108. Available from: https://doi.org/10.1016/j.pbi.2020.04.006

Fischer, C., DeFalco, T.A., Karia, P., Snedden, W.A., Moeder, W., Yoshioka, K. et al. (2017) Calmodulin as a Ca^{2+}-sensing subunit of Arabidopsis cyclic nucleotide-gated channel complexes. *Plant and Cell Physiology*, 58(7), 1208–1221. Available from: https://doi.org/10.1093/pcp/pcx052

Förderer, A., Li, E., Lawson, A.W., Deng, Y., Sun, Y., Logemann, E. et al. (2022) A wheat resistosome defines common principles of immune receptor channels. *Nature*, 610(7932), 532–539. Available from: https://doi.org/10.1038/s41586-022-05231-w

Fromm, H. & Chua, N.H. (1992) Cloning of plant cDNAs encoding calmodulin-binding proteins using 35S-labeled recombinant calmodulin as a probe. *Plant Molecular Biology Reporter*, 10(2), 199–206.

Galon, Y., Nave, R., Boyce, J.M., Nachmias, D., Knight, M.R. & Fromm, H. (2008) Calmodulin-binding transcription activator (CAMTA) 3 mediates biotic defense responses in Arabidopsis. *FEBS Letters*, 582(6), 943–948. Available from: https://doi.org/10.1016/j.febslet.2008.02.037

Gao, Q., Wang, C., Xi, Y., Shao, Q., Li, L. & Luan, S. (2022) A receptor-channel trio conducts Ca(2+) signalling for pollen tube reception. *Nature*, 607(7919), 534–539. Available from: https://doi.org/10.1038/s41586-022-04923-7

Gifford, J.L., Jamshidiha, M., Mo, J., Ishida, H. & Vogel, H.J. (2013) Comparing the calcium binding abilities of two soybean calmodulins: towards understanding the divergent nature of plant calmodulins. *The Plant Cell*, 25(11), 4512–4524. Available from: https://doi.org/10.1105/tpc.113.113183

Gifford, J.L., Walsh, M.P. & Vogel, H.J. (2007) Structures and metal-ion-binding properties of the Ca2+-binding helix-loop-helix EF-hand motifs. *Biochemical Journal*, 405(2), 199–221. Available from: https://doi.org/10.1042/BJ20070255

Gilroy, S., Białasek, M., Suzuki, N., Górecka, M., Devireddy, A.R., Karpiński, S. et al. (2016) ROS, calcium, and electric signals: key mediators of rapid systemic signaling in plants. *Plant Physiology*, 171(3), 1606–1615. Available from: https://doi.org/10.1104/pp.16.00434

Gut, H., Dominici, P., Pilati, S., Astegno, A., Petoukhov, M.V., Svergun, D.I. et al. (2009) A common structural basis for pH- and calmodulin-mediated regulation in plant glutamate decarboxylase. *Journal of Molecular Biology*, 392(2), 334–351. Available from: https://doi.org/10.1016/j.jmb.2009.06.080

Harding, S.A. (1997) Transgenic tobacco expressing a foreign calmodulin gene shows an enhanced production of active oxygen species. *The EMBO Journal*, 16(6), 1137–1144. Available from: https://doi.org/10.1093/emboj/16.6.1137

Harmoko, R., Fanata, W.I.D., Yoo, J.Y., Ko, K.S., Rim, Y.G., Uddin, M.N. et al. (2013) RNA-dependent RNA polymerase 6 is required for efficient hpRNA-induced gene silencing in plants. *Molecules and Cells*, 35(3), 202–209. Available from: https://doi.org/10.1007/s10059-013-2203-2

Heese, A., Hann, D.R., Gimenez-Ibanez, S., Jones, A.M.E., He, K. & Li, J. et al. (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 104(29), 12217–12222. Available from: https://doi.org/10.1073/pnas.0705306104

Heo, W.D., Lee, S.H., Kim, M.C., Kim, J.C., Chung, W.S. & Chun, H.J. et al. (1999) Involvement of specific calmodulin isoforms in salicylic acid-independent activation of plant disease resistance responses. *Proceedings of the National Academy of Sciences of the United States of America*, 96(2), 766–771. Available from: https://doi.org/10.1073/pnas.96.2.766

Heyer, M., Scholz, S.S., Reichelt, M., Kunert, G., Oelmüller, R. & Mithöfer, A. (2022) The Ca(2+) sensor proteins CML37 and CML42 antagonistically regulate plant stress responses by altering phytohormone signals. *Plant Molecular Biology*, 109(4–5), 611–625. Available from: https://doi.org/10.1007/s11103-021-01184-2

Hilleary, R. & Gilroy, S. (2018) Systemic signaling in response to wounding and pathogens. *Current Opinion in Plant Biology*, 43, 57–62. Available from: https://doi.org/10.1016/j.pbi.2017.12.009

Horsefield, S., Burdett, H., Zhang, X., Manik, M.K., Shi, Y., Chen, J. et al. (2019) NAD(+) cleavage activity by animal and plant TIR domains in cell death pathways. *Science*, 365(6455), 793–799. Available from: https://doi.org/10.1126/science.aax1911

Huang, W., Wu, Z., Tian, H., Li, X. & Zhang, Y. (2021) Arabidopsis CALMODULIN-BINDING PROTEIN 60b plays dual roles in plant immunity. *Plant Communications*, 2(6), 100213. Available from: https://doi.org/10.1016/j.xplc.2021.100213

Hussain, A.G., Wennmann, J.T., Goergen, G., Bryon, A. & Ros, V.I.D. (2021) Viruses of the fall armyworm *Spodoptera frugiperda*: a review with prospects for biological control. *Viruses*, 13(11), 2220. Available from: https://doi.org/10.3390/v13112220

Jacob, P., Kim, N.H., Wu, F., El-Kasmi, F., Chi, Y., Walton, W.G. et al. (2021) Plant "helper" immune receptors are Ca(2+)-permeable nonselective cation channels. *Science*, 373(6553), 420–425. Available from: https://doi.org/10.1126/science.abg7917

Jakoby, M., Weisshaar, B., Dröge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T. et al. (2002) bZIP transcription factors in Arabidopsis. *Trends in Plant Science*, 7(3), 106–111. Available from: https://doi.org/10.1016/s1360-1385(01)02223-3

Jarosch, B., Kogel, K.H. & Schaffrath, U. (1999) The ambivalence of the barley Mlo locus: mutations conferring resistance against powdery mildew (Blumeria graminis f. sp, hordei) enhance susceptibility to the rice blast fungus Magnaporthe grisea. *Molecular Plant-Microbe Interactions®*, 12(6), 508–514. Available from: https://doi.org/10.1094/Mpmi.1999.12.6.508

Jarratt-Barnham, E., Wang, L., Ning, Y. & Davies, J.M. (2021) The complex story of plant cyclic nucleotide-gated channels. *International Journal of Molecular Sciences*, 22(2), 874. Available from: https://doi.org/10.3390/ijms22020874

Jeon, E.J., Tadamura, K., Murakami, T., Inaba, J., Kim, B.M., Sato, M. et al. (2017) rgs-CaM detects and counteracts viral RNA silencing suppressors in plant immune priming. *Journal of Virology*, 91(19), e00761–00717. Available from: https://doi.org/10.1128/JVI.00761-17

Jeworutzki, E., Roelfsema, M.R.G., Anschütz, U., Krol, E., Elzenga, J.T.M., Felix, G. et al. (2010) Early signaling through the Arabidopsis pattern recognition receptors FLS2 and EFR involves Ca2+-associated opening of plasma membrane anion channels. *The Plant Journal*, 62(3), 367–378. Available from: https://doi.org/10.1111/j.1365-313X.2010.04155.x

Jiang, L., Anderson, J.C., Besteiro, M.A.G. & Peck, S.C. (2017) Phosphorylation of Arabidopsis MAP kinase phosphatase 1 (MKP1) is required for PAMP responses and resistance against bacteria. *Plant Physiology*, 175(4), 1839–1852. Available from: https://doi.org/10.1104/pp.17.01152

Jiang, S.C., Engle, N.L., Banday, Z.Z., Cecchini, N.M., Jung, H.W., Tschaplinski, T.J. et al. (2021) ALD1 accumulation in Arabidopsis epidermal plastids confers local and non-autonomous disease resistance. *Journal of Experimental Botany*, 72(7), 2710–2726. Available from: https://doi.org/10.1093/jxb/eraa609

Jiang, X., Hoehenwarter, W., Scheel, D. & Lee, J. (2020) Phosphorylation of the CAMTA3 transcription factor triggers its destabilization and nuclear export. *Plant Physiology*, 184(2), 1056–1071. Available from: https://doi.org/10.1104/pp.20.00795

Jiang, Y. & Ding, P. (2023) Calcium signaling in plant immunity: a spatiotemporally controlled symphony. *Trends in Plant Science*, 28(1), 74–89. Available from: https://doi.org/10.1016/j.tplants.2022.11.001

Jiao, C., Guo, Z., Gong, J., Zuo, Y., Li, S., Vanegas, D. et al. (2022) CML8 and GAD4 function in (Z)–3–hexenol–mediated defense by regulating γ–aminobutyric acid accumulation in Arabidopsis. *Plant Physiology and Biochemistry*, 186, 135–144. Available from: https://doi.org/10.1016/j.plaphy.2022.06.023

Jiao, C., Li, K., Zuo, Y., Gong, J., Guo, Z. & Shen, Y. (2022) CALMODULIN1 and WRKY53 function in plant defense by negatively regulating the jasmonic acid biosynthesis pathway in Arabidopsis. *International Journal of Molecular Sciences*, 23(14), 7718. Available from: https://doi.org/10.3390/ijms23147718

Journot-Catalino, N., Somssich, I.E., Roby, D. & Kroj, T. (2006) The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in *Arabidopsis thaliana*. *The Plant Cell*, 18(11), 3289–3302. Available from: https://doi.org/10.1105/tpc.106.044149

Jørgensen, I.H. (1992) Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica*, 63(1–2), 141–152.

Kadota, Y., Sklenar, J., Derbyshire, P., Stransfeld, L., Asai, S., Ntoukakis, V. et al. (2014) Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. *Molecular Cell*, 54(1), 43–55. Available from: https://doi.org/10.1016/j.molcel.2014.02.021

Kang, C.H., Jung, W.Y., Kang, Y.H., Kim, J.Y., Kim, D.G., Jeong, J.C. et al. (2006) AtBAG6, a novel calmodulin-binding protein, induces programmed cell death in yeast and plants. *Cell Death & Differentiation*, 13(1), 84–95. Available from: https://doi.org/10.1038/sj.cdd.4401712

Karita, E., Yamakawa, H., Mitsuhara, I., Kuchitsu, K. & Ohashi, Y. (2004) Three types of tobacco calmodulins characteristically activate plant NAD kinase at different Ca2+ concentrations and pHs. *Plant and Cell Physiology*, 45(10), 1371–1379. Available from: https://doi.org/10.1093/pcp/pch158

Katou, S., Kuroda, K., Seo, S., Yanagawa, Y., Tsuge, T., Yamazaki, M. et al. (2007) A calmodulin-binding mitogen-activated protein kinase phosphatase is induced by wounding and regulates the activities of stress-related mitogen-activated protein kinases in rice. *Plant and Cell Physiology*, 48(2), 332–344.

Keller, T., Damude, H.G., Werner, D., Doerner, P., Dixon, R.A. & Lamb, C. (1998) A plant homolog of the neutrophil NADPH oxidase gp91phox subunit gene encodes a plasma membrane protein with Ca2+ binding motifs. *The Plant Cell*, 10(2), 255–266.

Kesarwani, M., Yoo, J. & Dong, X. (2007) Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in Arabidopsis. *Plant Physiology*, 144(1), 336–346. Available from: https://doi.org/10.1104/pp.106.095299

Kim, D.S., Choi, H.W. & Hwang, B.K. (2014) Pepper mildew resistance locus O interacts with pepper calmodulin and suppresses Xanthomonas AvrBsT-triggered cell death and defense responses. *Planta*, 240(4), 827–839. Available from: https://doi.org/10.1007/s00425-014-2134-y

Kim, D.S. & Hwang, B.K. (2012) The pepper MLO gene, CaMLO2, is involved in the susceptibility cell-death response and bacterial and oomycete proliferation. *The Plant Journal*, 72(5), 843–855. Available from: https://doi.org/10.1111/tpj.12003

Kim, H.S., Park, B.O., Yoo, J.H., Jung, M.S., Lee, S.M., Han, H.J. et al. (2007) Identification of a calmodulin-binding NAC protein as a transcriptional repressor in Arabidopsis. *Journal of Biological Chemistry*, 282(50), 36292–36302. Available from: https://doi.org/10.1074/jbc.M705217200

Kim, H.S., Park, H.C., Kim, K.E., Jung, M.S., Han, H.J., Kim, S.H. et al. (2012) A NAC transcription factor and SNI1 cooperatively suppress basal pathogen resistance in *Arabidopsis thaliana*. *Nucleic Acids Research*, 40(18), 9182–9192. Available from: https://doi.org/10.1093/nar/gks683

Kim, K.C., Fan, B. & Chen, Z. (2006) Pathogen-induced Arabidopsis WRKY7 is a transcriptional repressor and enhances plant susceptibility to Pseudomonas syringae. *Plant Physiology*, 142(3), 1180–1192. Available from: https://doi.org/10.1104/pp.106.082487

Kim, M.C., Lee, S.H., Kim, J.K., Chun, H.J., Choi, M.S., Chung, W.S. et al. (2002) Mlo, a modulator of plant defense and cell death, is a novel calmodulin-binding protein: Isolation and characterization of a rice Mlo homologue. *Journal of Biological Chemistry*, 277(22), 19304–19314. Available from: https://doi.org/10.1074/jbc.M108478200

Kim, M.C., Panstruga, R., Elliott, C., Müller, J., Devoto, A., Yoon, H.W. et al. (2002) Calmodulin interacts with MLO protein to regulate defence against mildew in barley. *Nature*, 416(6879), 447–451. Available from: https://doi.org/10.1038/416447a

Kim, Y., Gilmour, S.J., Chao, L., Park, S. & Thomashow, M.F. (2020) Arabidopsis CAMTA transcription factors regulate pipecolic acid biosynthesis and priming of immunity genes. *Molecular Plant*, 13(1), 157–168. Available from: https://doi.org/10.1016/j.molp.2019.11.001

Konishi, S., Sasakuma, T. & Sasanuma, T. (2010) Identification of novel Mlo family members in wheat and their genetic characterization. *Genes & Genetic Systems*, 85(3), 167–175. Available from: https://doi.org/10.1266/ggs.85.167

Koo, S.C., Choi, M.S., Chun, H.J., Shin, D.B., Park, B.S., Kim, Y.H. et al. (2009) The calmodulin-binding transcription factor OsCBT suppresses defense responses to pathogens in rice. *Molecules and Cells*, 27(5), 563–570. Available from: https://doi.org/10.1007/s10059-009-0081-4

Köster, P., DeFalco, T.A. & Zipfel, C. (2022) Ca(2+) signals in plant immunity. *The EMBO Journal*, 41(12), e110741. Available from: https://doi.org/10.15252/embj.2022110741

Kudla, J., Batistič, O. & Hashimoto, K. (2010) Calcium signals: the lead currency of plant information processing. *The Plant Cell*, 22(3), 541–563. Available from: https://doi.org/10.1105/tpc.109.072686

Kumar, J., Hückelhoven, R., Beckhove, U., Nagarajan, S. & Kogel, K.H. (2001) A compromised Mlo pathway affects the response of barley to the necrotrophic fungus *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) and its toxins. *Phytopathology®*, 91(2), 127–133. Available from: https://doi.org/10.1094/PHYTO.2001.91.2.127

Kumar, S., Mazumder, M., Gupta, N., Chattopadhyay, S. & Gourinath, S. (2016) Crystal structure of *Arabidopsis thaliana* calmodulin7 and insight into its mode of DNA binding. *FEBS Letters*, 590(17), 3029–3039. Available from: https://doi.org/10.1002/1873-3468.12349

Laluk, K., Prasad, K.V.S.K., Savchenko, T., Celesnik, H., Dehesh, K., Levy, M. et al. (2012) The calmodulin-binding transcription factor SIGNAL RESPONSIVE1 is a novel regulator of glucosinolate metabolism and herbivory tolerance in Arabidopsis. *Plant and Cell Physiology*, 53(12), 2008–2015. Available from: https://doi.org/10.1093/pcp/pcs143

Leba, L.J., Cheval, C., Ortiz-Martín, I., Ranty, B., Beuzón, C.R., Galaud, J.P. et al. (2012) CML9, an Arabidopsis calmodulin-like protein, contributes to plant innate immunity through a flagellin-dependent signalling pathway. *The Plant Journal*, 71(6), 976–989. Available from: https://doi.org/10.1111/j.1365-313X.2012.05045.x

Lecourieux, D., Mazars, C., Pauly, N., Ranjeva, R. & Pugin, A. (2002) Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells. *The Plant Cell*, 14(10), 2627–2641.

Lee, D., Lal, N.K., Lin, Z.-J.D., Ma, S., Liu, J., Castro, B. et al. (2020) Regulation of reactive oxygen species during plant immunity through phosphorylation and ubiquitination of RBOHD. *Nature Communications*, 11(1), 1838. Available from: https://doi.org/10.1038/s41467-020-15601-5

Lee, K., Song, E.H., Kim, H.S., Yoo, J.H., Han, H.J., Jung, M.S. et al. (2008) Regulation of MAPK phosphatase 1 (AtMKP1) by calmodulin in Arabidopsis. *Journal of Biological Chemistry*, 283(35), 23581–23588.

Lee, S.H., Johnson, J.D., Walsh, M.P., Van Lierop, J.E., Sutherland, C., Xu, A. et al. (2000) Differential regulation of Ca2+/calmodulin-dependent enzymes by plant calmodulin isoforms and free Ca2+ concentration. *Biochemical Journal*, 350, 299–306.

Levy, M., Wang, Q., Kaspi, R., Parrella, M.P. & Abel, S. (2005) Arabidopsis IQD1, a novel calmodulin-binding nuclear protein, stimulates glucosinolate accumulation and plant defense. *The Plant Journal*, 43(1), 79–96.

Li, L., Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z. et al. (2014) The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. *Cell Host & Microbe*, 15(3), 329–338. Available from: https://doi.org/10.1016/j.chom.2014.02.009

Li, L.S., Ying, J., Li, E., Ma, T., Li, M., Gong, L.M. et al. (2021) Arabidopsis CBP60b is a central transcriptional activator of immunity. *Plant Physiology*, 186(3), 1645–1659. Available from: https://doi.org/10.1093/plphys/kiab164

Li, X., Huang, L., Zhang, Y., Ouyang, Z., Hong, Y., Zhang, H. et al. (2014) Tomato SR/CAMTA transcription factors SlSR1 and SlSR3L negatively regulate disease resistance response and SlSR1L positively modulates drought stress tolerance. *BMC Plant Biology*, 14(286), 286. Available from: https://doi.org/10.1186/s12870-014-0286-3

Li, Y., Kabbage, M., Liu, W. & Dickman, M.B. (2016) Aspartyl protease-mediated cleavage of BAG6 is necessary for autophagy and fungal resistance in plants. *The Plant Cell*, 28(1), 233–247. Available from: https://doi.org/10.1105/tpc.15.00626

Liu, Q. & Zhu, H. (2008) Molecular evolution of the MLO gene family in *Oryza sativa* and their functional divergence. *Gene*, 409(1–2), 1–10. Available from: https://doi.org/10.1016/j.gene.2007.10.031

Liu, Y., Chen, W., Liu, L., Su, Y., Li, Y. & Jia, W. et al. (2022) Genome-wide identification and expression analysis of calmodulin and calmodulin-like genes in wheat (*Triticum aestivum* L.). *Plant Signaling & Behavior*, 17(1), 2013646. Available from: https://doi.org/10.1080/15592324.2021.2013646

Lolle, S., Greeff, C., Petersen, K., Roux, M., Jensen, M.K., Bressendorff, S. et al. (2017) Matching NLR immune receptors to autoimmunity in camta3 mutants using antimorphic NLR alleles. *Cell Host & Microbe*, 21(4), 518–529. Available from: https://doi.org/10.1016/j.chom.2017.03.005

Lu, L., Rong, W., Zhou, R., Huo, N. & Zhang, Z. (2019) TaCML36, a wheat calmodulin-like protein, positively participates in an immune response to Rhizoctonia cerealis. *The Crop Journal*, 7(5), 608–618. Available from: https://doi.org/10.1016/j.cj.2019.02.001

Lu, Y., Truman, W., Liu, X., Bethke, G., Zhou, M., Myers, C.L. et al. (2018) Different modes of negative regulation of plant immunity by calmodulin-related genes. *Plant Physiology*, 176(4), 3046–3061. Available from: https://doi.org/10.1104/pp.17.01209

Luan, S. & Wang, C. (2021) Calcium signaling mechanisms across kingdoms. *Annual Review of Cell and Developmental Biology*, 37(1), 311–340. Available from: https://doi.org/10.1146/annurev-cellbio-120219-035210

Ma, S., Lapin, D., Liu, L., Sun, Y., Song, W. & Zhang, X. et al. (2020) Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. *Science*, 370(6521), eabe3069. Available from: https://doi.org/10.1126/science.abe3069

Ma, W., Smigel, A., Tsai, Y.C., Braam, J. & Berkowitz, G.A. (2008) Innate immunity signaling: cytosolic Ca2+ elevation is linked to downstream nitric oxide generation through the action of calmodulin or a calmodulin-like protein. *Plant Physiology*, 148(2), 818–828. Available from: https://doi.org/10.1104/pp.108.125104

MacGregor, K.B., Shelp, B.J., Peiris, S. & Bown, A.W. (2003) Over-expression of glutamate decarboxylase in transgenic tobacco plants deters feeding by phytophagous insect larvae. *Journal of Chemical Ecology*, 29(9), 2177–2182.

Makiyama, R.K., Fernandes, C.A.H., Dreyer, T.R., Moda, B.S., Matioli, F.F., Fontes, M.R.M. et al. (2016) Structural and thermodynamic studies of the tobacco calmodulin-like rgs-CaM protein. *International Journal of Biological Macromolecules*, 92, 1288–1297. Available from: https://doi.org/10.1016/j.ijbiomac.2016.08.016

Marcec, M.J., Gilroy, S., Poovaiah, B.W. & Tanaka, K. (2019) Mutual interplay of Ca2+ and ROS signaling in plant immune response. *Plant Science*, 283, 343–354. Available from: https://doi.org/10.1016/j.plantsci.2019.03.004

McCormack, E. & Braam, J. (2003) Calmodulins and related potential calcium sensors of Arabidopsis. *New Phytologist*, 159(3), 585–598. Available from: https://doi.org/10.1046/j.1469-8137.2003.00845.x

McLean, M.D. (2003) Overexpression of glutamate decarboxylase in transgenic tobacco plants confers resistance to the northern root-knot nematode. *Molecular Breeding*, 11(4), 277–285. Available from: https://doi.org/10.1023/A:1023483106582

Meena, M.K., Prajapati, R., Krishna, D., Divakaran, K., Pandey, Y., Reichelt, M. et al. (2019) The Ca(2+) channel CNGC19 regulates arabidopsis defense against spodoptera herbivory. *The Plant Cell*, 31(7), 1539–1562. Available from: https://doi.org/10.1105/tpc.19.00057

Meng, X. & Zhang, S. (2013) MAPK cascades in plant disease resistance signaling. *Annual Review of Phytopathology*, 51, 245–266. Available from: https://doi.org/10.1146/annurev-phyto-082712-102314

Moeder, W., Phan, V. & Yoshioka, K. (2019) Ca2+ to the rescue – Ca2+channels and signaling in plant immunity. *Plant Science*, 279, 19–26. Available from: https://doi.org/10.1016/j.plantsci.2018.04.012

Mohanta, T.K., Kumar, P. & Bae, H. (2017) Genomics and evolutionary aspect of calcium signaling event in calmodulin and calmodulin-like proteins in plants. *BMC Plant Biology*, 17(1), 38. Available from: https://doi.org/10.1186/s12870-017-0989-3

Munir, S., Khan, M.R.G., Song, J., Munir, S., Zhang, Y., Ye, Z. et al. (2016) Genome-wide identification, characterization and expression analysis of calmodulin-like (CML) proteins in tomato (*Solanum lycopersicum*). *Plant Physiology and Biochemistry*, 102, 167–179. Available from: https://doi.org/10.1016/j.plaphy.2016.02.020

Murray, S.L., Ingle, R.A., Petersen, L.N. & Denby, K.J. (2007) Basal resistance against Pseudomonas syringae in Arabidopsis involves WRKY53 and a protein with homology to a nematode resistance protein. *Molecular Plant-Microbe Interactions®*, 20(11), 1431–1438.

Nakahara, K.S., Masuta, C., Yamada, S., Shimura, H., Kashihara, Y. & Wada, T.S. et al. (2012) Tobacco calmodulin-like protein provides secondary defense by binding to and directing degradation of virus RNA silencing suppressors. *Proceedings of the National Academy of Sciences of the United States of America*, 109(25), 10113–10118. Available from: https://doi.org/10.1073/pnas.1201628109

Nie, H., Zhao, C., Wu, G., Wu, Y., Chen, Y. & Tang, D. (2012) SR1, a calmodulin-binding transcription factor, modulates plant defense and ethylene-induced senescence by directly regulating NDR1 and EIN3. *Plant Physiology*, 158(4), 1847–1859. Available from: https://doi.org/10.1104/pp.111.192310

Nühse, T.S., Bottrill, A.R., Jones, A.M.E. & Peck, S.C. (2007) Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. *The Plant Journal*, 51(5), 931–940. Available from: https://doi.org/10.1111/j.1365-313X.2007.03192.x

Ogasawara, Y., Kaya, H., Hiraoka, G., Yumoto, F., Kimura, S., Kadota, Y. et al. (2008) Synergistic activation of the Arabidopsis NADPH oxidase AtrbohD by Ca2+ and phosphorylation. *Journal of Biological Chemistry*, 283(14), 8885–8892. Available from: https://doi.org/10.1074/jbc.M708106200

Ogunrinde, A., Munro, K., Davidson, A., Ubaid, M. & Snedden, W.A. (2017) *Arabidopsis* calmodulin-like proteins, CML15 and CML16 possess biochemical properties distinct from calmodulin and show non-overlapping tissue expression patterns. *Frontiers in Plant Science*, 8(2175), 2175. Available from: https://doi.org/10.3389/fpls.2017.02175

Oka, K., Amano, Y., Katou, S., Seo, S., Kawazu, K., Mochizuki, A. et al. (2013) Tobacco MAP kinase phosphatase (NtMKP1) negatively regulates wound response and induced resistance against necrotrophic pathogens and lepidopteran herbivores. *Molecular Plant-Microbe Interactions®*, 26(6), 668–675. Available from: https://doi.org/10.1094/MPMI-11-12-0272-R

Panstruga, R. (2005a) Discovery of novel conserved peptide domains by ortholog comparison within plant multi-protein families. *Plant Molecular Biology*, 59(3), 485–500. Available from: https://doi.org/10.1007/s11103-005-0353-0

Panstruga, R. (2005b) Serpentine plant MLO proteins as entry portals for powdery mildew fungi. *Biochemical Society Transactions*, 33(2), 289–392.

Park, C.Y., Lee, J.H., Yoo, J.H., Moon, B.C., Choi, M.S., Kang, Y.H. et al. (2005) WRKY group IId transcription factors interact with calmodulin. *FEBS Letters*, 579(6), 1545–1550. Available from: https://doi.org/10.1016/j.febslet.2005.01.057

Park, H.C., Kim, M.L., Kang, Y.H., Jeon, J.M., Yoo, J.H., Kim, M.C. et al. (2004) Pathogen- and NaCl-induced expression of the SCaM-4 promoter is mediated in part by a GT-1 box that interacts with a GT-1-like transcription factor. *Plant Physiology*, 135(4), 2150–2161. Available from: https://doi.org/10.1104/pp.104.041442

Peng, H., Yang, T. & Ii, W. (2014) Calmodulin gene expression in response to mechanical wounding and *Botrytis cinerea* infection in tomato fruit. *Plants*, 3(3), 427–441. Available from: https://doi.org/10.3390/plants3030427

Poovaiah, B.W., Du, L., Wang, H. & Yang, T. (2013) Recent advances in calcium/calmodulin-mediated signaling with an emphasis on plant-microbe interactions. *Plant Physiology*, 163(2), 531–542. Available from: https://doi.org/10.1104/pp.113.220780

Popescu, S.C., Popescu, G.V., Bachan, S., Zhang, Z.M., Seay, M. & Gerstein, M. et al. (2007) Differential binding of calmodulin-related proteins to their targets revealed through high-density Arabidopsis protein microarrays. *Proceedings of the National Academy of Sciences of the United States of America*, 104(11), 4730–4735. Available from: https://doi.org/10.1073/pnas.0611615104

Qin, J., Wang, K., Sun, L., Xing, H., Wang, S. & Li, L. et al. (2018) The plant-specific transcription factors CBP60g and SARD1 are targeted by a Verticillium secretory protein VdSCP41 to modulate immunity. *eLife*, 7, e34902. Available from: https://doi.org/10.7554/eLife.34902

Qiu, Y., Xi, J., Du, L., Suttle, J.C. & Poovaiah, B.W. (2012) Coupling calcium/calmodulin-mediated signaling and herbivore-induced plant response through calmodulin-binding transcription factor AtSR1/CAMTA3. *Plant Molecular Biology*, 79(1–2), 89–99. Available from: https://doi.org/10.1007/s11103-012-9896-z

Rahman, H., Xu, Y.P., Zhang, X.R. & Cai, X.Z. (2016) *Brassica napus* genome possesses extraordinary high number of CAMTA genes and CAMTA3 contributes to PAMP triggered immunity and resistance to Sclerotinia sclerotiorum. *Frontiers in Plant Science*, 7, 581. Available from: https://doi.org/10.3389/fpls.2016.00581

Ranf, S., Eschen-Lippold, L., Pecher, P., Lee, J. & Scheel, D. (2011) Interplay between calcium signalling and early signalling elements during defence responses to microbe- or damage-associated molecular patterns. *The Plant Journal*, 68(1), 100–113. Available from: https://doi.org/10.1111/j.1365-313X.2011.04671.x

Rao, S.S., El-Habbak, M.H., Havens, W.M., Singh, A., Zheng, D., Vaughn, L. et al. (2014) Overexpression of GmCaM4 in soybean enhances resistance to pathogens and tolerance to salt stress. *Molecular Plant Pathology*, 15(2), 145–160. Available from: https://doi.org/10.1111/mpp.12075

Reddy, A.S.N., Ali, G.S., Celesnik, H. & Day, I.S. (2011) Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. *The Plant Cell*, 23(6), 2010–2032. Available from: https://doi.org/10.1105/tpc.111.084988

Reddy, A.S.N., Takezawa, D., Fromm, H. & Poovaiah, B.W. (1993) Isolation and characterization of two cDNAs that encode for calmodulin-binding proteins from corn root tips. *Plant Science*, 94(1–2), 109–117.

Reddy, V.S., Ali, G.S. & Reddy, A.S.N. (2002) Genes encoding calmodulin-binding proteins in the Arabidopsis genome. *Journal of Biological Chemistry*, 277(12), 9840–9852. Available from: https://doi.org/10.1074/jbc.M111626200

Rentel, M.C. & Knight, M.R. (2004) Oxidative stress-induced calcium signaling in Arabidopsis. *Plant Physiology*, 135(3), 1471–1479. Available from: https://doi.org/10.1104/pp.104.042663

Robatzek, S., Chinchilla, D. & Boller, T. (2006) Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis. *Genes & Development*, 20(5), 537–542. Available from: https://doi.org/10.1101/gad.366506

Roux, M., Schwessinger, B., Albrecht, C., Chinchilla, D., Jones, A., Holton, N. et al. (2011) The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *The Plant Cell*, 23(6), 2440–2455. Available from: https://doi.org/10.1105/tpc.111.084301

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N. & Nelson, A. (2019) The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 3(3), 430–439. Available from: https://doi.org/10.1038/s41559-018-0793-y

Scholz, S.S., Vadassery, J., Heyer, M., Reichelt, M., Bender, K.W., Snedden, W.A. et al. (2014) Mutation of the Arabidopsis calmodulin-like protein CML37 deregulates the jasmonate pathway and enhances susceptibility to herbivory. *Molecular Plant*, 7(12), 1712–1726. Available from: https://doi.org/10.1093/mp/ssu102

Shen, L., Yang, S., Guan, D. & He, S. (2020) CaCML13 acts positively in pepper immunity against ralstonia solanacearum infection forming feedback loop with CabZIP63. *International Journal of Molecular Sciences*, 21(11), 4186. Available from: https://doi.org/10.3390/ijms21114186

Snedden, W.A., Koutsia, N., Baum, G. & Fromm, H. (1996) Activation of a recombinant petunia glutamate decarboxylase by calcium/calmodulin or by a monoclonal antibody which recognizes the calmodulin binding domain. *Journal of Biological Chemistry*, 271(8), 4148–4153.

Stein, M., Dittgen, J., Sánchez-Rodríguez, C., Hou, B.H., Molina, A., Schulze-Lefert, P. et al. (2006) Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. *The Plant Cell*, 18(3), 731–746. Available from: https://doi.org/10.1105/tpc.105.038372

Stokstad, E. (2017) New crop pest takes Africa at lightning speed. *Science*, 356(6337), 473–474. Available from: https://doi.org/10.1126/science.356.6337.473

Sun, L., Qin, J., Wu, X., Zhang, J. & Zhang, J. (2022) TOUCH 3 and CALMODULIN 1/4/6 cooperate with calcium-dependent protein kinases to trigger calcium-dependent activation of CAM-BINDING PROTEIN 60-LIKE G and regulate fungal resistance in plants. *The Plant Cell*, 34(10), 4088–4104. Available from: https://doi.org/10.1093/plcell/koac209

Sun, T., Huang, J., Xu, Y., Verma, V., Jing, B., Sun, Y. et al. (2020) Redundant CAMTA transcription factors negatively regulate the biosynthesis of salicylic acid and N-hydroxypipecolic acid by modulating the expression of SARD1 and CBP60g. *Molecular Plant*, 13(1), 144–156. Available from: https://doi.org/10.1016/j.molp.2019.10.016

Sun, T., Zhang, Y., Li, Y., Zhang, Q., Ding, Y. & Zhang, Y. (2015) ChIP-seq reveals broad roles of SARD1 and CBP60g in regulating plant immunity. *Nature Communications*, 6, 10159. Available from: https://doi.org/10.1038/ncomms10159

Sun, X., Lapin, D., Feehan, J.M., Stolze, S.C., Kramer, K., Dongus, J.A. et al. (2021) Pathogen effector recognition-dependent association of NRG1 with EDS1 and SAG101 in TNL receptor immunity. *Nature Communications*, 12(1), 3335. Available from: https://doi.org/10.1038/s41467-021-23614-x

Szymanski, D.B., Liao, B., Zielinski, R.E., Szymanski, D.B., Liao, B. & Zielinski, R.E. (1996) Calmodulin isoforms differentially enhance the binding of cauliflower nuclear proteins and recombinant TGA3 to a region derived from the Arabidopsis Cam-3 promoter. *The Plant Cell*, 8, 1069–1077.

Takabatake, R., Karita, E., Seo, S., Mitsuhara, I., Kuchitsu, K. & Ohashi, Y. (2007) Pathogen-induced calmodulin isoforms in basal resistance against bacterial and fungal pathogens in tobacco. *Plant and Cell Physiology*, 48(3), 414–423. Available from: https://doi.org/10.1093/pcp/pcm011

Takahashi, F., Mizoguchi, T., Yoshida, R., Ichimura, K. & Shinozaki, K. (2011) Calmodulin-dependent activation of MAP kinase for ROS homeostasis in Arabidopsis. *Molecular Cell*, 41(6), 649–660. Available from: https://doi.org/10.1016/j.molcel.2011.02.029

Tarkowski, Ł.P., Signorelli, S. & Höfte, M. (2020) γ-Aminobutyric acid and related amino acids in plant immune responses: emerging mechanisms of action. *Plant, Cell & Environment*, 43(5), 1103–1116. Available from: https://doi.org/10.1111/pce.13734

Teresinski, H.J., Hau, B., Symonds, K., Kilburn, R., Munro, K.A., Doner, N.M. et al. (2023) Arabidopsis calmodulin-like proteins CML13 and CML14 interact with proteins that have IQ domains. *Plant, Cell & Environment*, 46, 2470–2491. Available from: https://doi.org/10.1111/pce.14616

Tian, W., Hou, C., Ren, Z., Wang, C., Zhao, F., Dahlbeck, D. et al. (2019) A calmodulin-gated calcium channel links pathogen patterns to plant immunity. *Nature*, 572(7767), 131–135. Available from: https://doi.org/10.1038/s41586-019-1413-y

Truman, W., Sreekanta, S., Lu, Y., Bethke, G., Tsuda, K., Katagiri, F. et al. (2013) The CALMODULIN-BINDING PROTEIN60 family includes both negative and positive regulators of plant immunity. *Plant Physiology*, 163(4), 1741–1751. Available from: https://doi.org/10.1104/pp.113.227108

Turner, W.L., Waller, J.C., Vanderbeld, B. & Snedden, W.A. (2004) Cloning and characterization of two NAD kinases from Arabidopsis. Identification of a calmodulin binding isoform. *Plant Physiology*, 135(3), 1243–1255.

Urquhart, W., Gunawardena, A.H.L.A.N., Moeder, W., Ali, R., Berkowitz, G.A. & Yoshioka, K. (2007) The chimeric cyclic nucleotide-gated ion channel ATCNGC11/12 constitutively induces programmed cell death in a Ca2+ dependent manner. *Plant Molecular Biology*, 65(6), 747–761. Available from: https://doi.org/10.1007/s11103-007-9239-7

Vadassery, J., Reichelt, M., Hause, B., Gershenzon, J., Boland, W. & Mithöfer, A. (2012) CML42-mediated calcium signaling coordinates responses to Spodoptera herbivory and abiotic stresses in Arabidopsis. *Plant Physiology*, 159(3), 1159–1175. Available from: https://doi.org/10.1104/pp.112.198150

Vallone, R., La Verde, V., D'Onofrio, M., Giorgetti, A., Dominici, P. & Astegno, A. (2016) Metal binding affinity and structural properties of calmodulin-like protein 14 from *Arabidopsis thaliana*. *Protein Science*, 25(8), 1461–1471. Available from: https://doi.org/10.1002/pro.2942

Vanderbeld, B. & Snedden, W.A. (2007) Developmental and stimulus-induced expression patterns of Arabidopsis calmodulin-like genes CML37, CML38 and CML39. *Plant Molecular Biology*, 64(6), 683–697. Available from: https://doi.org/10.1007/s11103-007-9189-0

La Verde, V., Dominici, P. & Astegno, A. (2018) Towards understanding plant calcium signaling through calmodulin-like proteins: a biochemical and structural perspective. *International Journal of Molecular Sciences*, 19(5), 1331.

Wan, D., Li, R., Zou, B., Zhang, X., Cong, J., Wang, R. et al. (2012) Calmodulin-binding protein CBP60g is a positive regulator of both disease resistance and drought tolerance in Arabidopsis. *Plant Cell Reports*, 31(7), 1269–1281. Available from: https://doi.org/10.1007/s00299-012-1247-7

Wang, G., Zeng, H., Hu, X., Zhu, Y., Chen, Y., Shen, C. et al. (2015) Identification and expression analyses of calmodulin-binding transcription activator genes in soybean. *Plant and Soil*, 386(1–2), 205–221. Available from: https://doi.org/10.1007/s11104-014-2267-6

Wang, L., Tsuda, K., Sato, M., Cohen, J.D., Katagiri, F. & Glazebrook, J. (2009) Arabidopsis CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against Pseudomonas syringae. *PLoS Pathogens*, 5(2), e1000301.

Wang, L., Tsuda, K., Truman, W., Sato, M., Nguyen, L.V., Katagiri, F. et al. (2011) CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling. *The Plant Journal*, 67(6), 1029–1041. Available from: https://doi.org/10.1111/j.1365-313X.2011.04655.x

Wang, Q., Shen, T., Ni, L., Chen, C., Jiang, J., Cui, Z. et al. (2023) Phosphorylation of OsRbohB by the protein kinase OsDMI3 promotes H(2)O(2) production to potentiate ABA responses in rice. *Molecular Plant*, 16(5), 882–902. Available from: https://doi.org/10.1016/j.molp.2023.04.003

Wang, W., Qin, L., Zhang, W., Tang, L., Zhang, C., Dong, X. et al. (2023) WeiTsing, a pericycle-expressed ion channel, safeguards the stele to confer clubroot resistance. *Cell*, 186(12), 2656–2671. Available from: https://doi.org/10.1016/j.cell.2023.05.023

Wang, Y., Gong, Q., Wu, Y., Huang, F., Ismayil, A., Zhang, D. et al. (2021) A calmodulin-binding transcription factor links calcium signaling to antiviral RNAi defense in plants. *Cell Host & Microbe*, 29(9), 1393–1406. Available from: https://doi.org/10.1016/j.chom.2021.07.003

Wang, Y., Pruitt, R.N., Nürnberger, T. & Wang, Y. (2022) Evasion of plant immunity by microbial pathogens. *Nature Reviews Microbiology*, 20(8), 449–464. Available from: https://doi.org/10.1038/s41579-022-00710-3

Wang, Y., Wei, F., Zhou, H., Liu, N., Niu, X., Yan, C. et al. (2019) TaCAMTA4, a calmodulin-interacting protein, involved in defense response of wheat to *Puccinia triticina*. *Scientific Reports*, 9(1), 641. Available from: https://doi.org/10.1038/s41598-018-36385-1

Wani, S.H., Anand, S., Singh, B., Bohra, A. & Joshi, R. (2021) WRKY transcription factors and plant defense responses: latest discoveries and future prospects. *Plant Cell Reports*, 40(7), 1071–1085. Available from: https://doi.org/10.1007/s00299-021-02691-8

Waszczak, C., Carmody, M. & Kangasjärvi, J. (2018) Reactive oxygen species in plant signaling. *Annual review of plant biology*, 69, 209–236. Available from: https://doi.org/10.1146/annurev-arplant-042817-040322

Webb, A.A.R., McAinsh, M.R., Taylor, J.E. & Hetherington, A.M. (1996) Calcium ions as intracellular second messengers in higher plants. *Advances in Botanical Research*, 22(8), 45–96. Available from: https://doi.org/10.1016/S0065-2296(08)60056-7

Wu, F., Chi, Y., Jiang, Z., Xu, Y., Xie, L., Huang, F. et al. (2020) Hydrogen peroxide sensor HPCA1 is an LRR receptor kinase in Arabidopsis. *Nature*, 578(7796), 577–581. Available from: https://doi.org/10.1038/s41586-020-2032-3

Wu, Z., Tian, L., Liu, X., Zhang, Y. & Li, X. (2021) TIR signal promotes interactions between lipase-like proteins and ADR1-L1 receptor and ADR1-L1 oligomerization. *Plant Physiology*, 187(2), 681–686. Available from: https://doi.org/10.1093/plphys/kiab305

Xiao, P., Feng, J.W., Zhu, X.T. & Gao, J. (2021) Evolution analyses of CAMTA transcription factor in plants and its enhancing effect on cold-tolerance. *Frontiers in Plant Science*, 12(2494), 758187. Available from: https://doi.org/10.3389/fpls.2021.758187

Xing, T., Higgins, V.J. & Blumwald, E. (1997) Race-specific elicitors of Cladosporium fulvum promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells. *The Plant Cell*, 9(2), 249–259.

Xu, B., Cheval, C., Laohavisit, A., Hocking, B., Chiasson, D., Olsson, T.S.G. et al. (2017) A calmodulin-like protein regulates plasmodesmal closure during bacterial immune responses. *New Phytologist*, 215(1), 77–84. Available from: https://doi.org/10.1111/nph.14599

Xu, G., Moeder, W., Yoshioka, K. & Shan, L. (2022) A tale of many families: calcium channels in plant immunity. *The Plant Cell*, 34(5), 1551–1567. Available from: https://doi.org/10.1093/plcell/koac033

Yamakawa, H., Katou, S., Seo, S., Mitsuhara, I., Kamada, H. & Ohashi, Y. (2004) Plant MAPK phosphatase interacts with calmodulins. *Journal*

of Biological Chemistry, 279(2), 928–936. Available from: https://doi.org/10.1074/jbc.M310277200

Yamakawa, H., Mitsuhara, I., Ito, N., Seo, S., Kamada, H. & Ohashi, Y. (2001) Transcriptionally and post-transcriptionally regulated response of 13 calmodulin genes to tobacco mosaic virus-induced cell death and wounding in tobacco plant. *European Journal of Biochemistry*, 268(14), 3916–3929.

Yamniuk, A.P. & Vogel, H.J. (2005) Structural investigation into the differential target enzyme regulation displayed by plant calmodulin isoforms. *Biochemistry*, 44(8), 3101–3111.

Yang, D.L., Shi, Z., Bao, Y., Yan, J., Yang, Z., Yu, H. et al. (2017) Calcium pumps and interacting BON1 protein modulate calcium signature, stomatal closure, and plant immunity. *Plant Physiology*, 175(1), 424–437. Available from: https://doi.org/10.1104/pp.17.00495

Yang, T. & Poovaiah, B.W. (2000a) An early ethylene up-regulated gene encoding a calmodulin-binding protein involved in plant senescence and death. *Journal of Biological Chemistry*, 275(49), 38467–38473. Available from: https://doi.org/10.1074/jbc.M003566200

Yang, T. & Poovaiah, B.W. (2000b) Molecular and biochemical evidence for the involvement of calcium/calmodulin in auxin action. *Journal of Biological Chemistry*, 275(5), 3137–3143.

Yang, T. & Poovaiah, B.W. (2002a) A calmodulin-binding/CGCG box DNA-binding protein family involved in multiple signaling pathways in plants. *Journal of Biological Chemistry*, 277(47), 45049–45058. Available from: https://doi.org/10.1074/jbc.M207941200

Yang, T. & Poovaiah, B.W. (2002b) Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin. *Proceedings of the National Academy of Science of the United States of America*, 99(6), 4097–4102. Available from: https://doi.org/10.1073/pnas.052564899

Ye, J., Zhang, L., Zhang, X., Wu, X. & Fang, R. (2021) Plant defense networks against insect-borne pathogens. *Trends in Plant Science*, 26(3), 272–287. Available from: https://doi.org/10.1016/j.tplants.2020.10.009

Yoo, J.H., Park, C.Y., Kim, J.C., Do Heo, W., Cheong, M.S., Park, H.C. et al. (2005) Direct interaction of a divergent CaM isoform and the transcription factor, MYB2, enhances salt tolerance in arabidopsis. *Journal of Biological Chemistry*, 280(5), 3697–3706. Available from: https://doi.org/10.1074/jbc.M408237200

Yoshioka, K., Moeder, W., Kang, H.G., Kachroo, P., Masmoudi, K., Berkowitz, G. et al. (2006) The chimeric Arabidopsis CYCLIC NUCLEOTIDE-GATED ION CHANNEL11/12 activates multiple pathogen resistance responses. *The Plant Cell*, 18(3), 747–763. Available from: https://doi.org/10.1105/tpc.105.038786

Yu, G., Wang, X., Chen, Q., Cui, N., Yu, Y. & Fan, H. (2019) Cucumber mildew resistance locus O interacts with calmodulin and regulates plant cell death associated with plant immunity. *International Journal of Molecular Sciences*, 20(12), 2995. Available from: https://doi.org/10.3390/ijms20122995

Yu, X., Feng, B., He, P. & Shan, L. (2017) From chaos to harmony: responses and signaling upon microbial pattern recognition. *Annual Review of Phytopathology*, 55, 109–137. Available from: https://doi.org/10.1146/annurev-phyto-080516-035649

Yu, X., Xu, G., Li, B., de Souza Vespoli, L., Liu, H., Moeder, W. et al. (2019) The receptor kinases BAK1/SERK4 regulate Ca(2+) channel-mediated cellular homeostasis for cell death containment. *Current Biology*, 29(22), 3778–3790. Available from: https://doi.org/10.1016/j.cub.2019.09.018

Yuan, H.M., Liu, W.C. & Lu, Y.T. (2017) CATALASE2 coordinates SA-mediated repression of both auxin accumulation and JA biosynthesis in plant defenses. *Cell Host & Microbe*, 21(2), 143–155. Available from: https://doi.org/10.1016/j.chom.2017.01.007

Yuan, P., Tanaka, K. & Poovaiah, B.W. (2021) Calmodulin-binding transcription activator AtSR1/CAMTA3 fine-tunes plant immune response by transcriptional regulation of the salicylate receptor NPR1. *Plant, Cell & Environment*, 44, 3140–3154. Available from: https://doi.org/10.1111/pce.14123

Zeng, H., Xu, L., Singh, A., Wang, H., Du, L. & Poovaiah, B.W. (2015) Involvement of calmodulin and calmodulin-like proteins in plant responses to abiotic stresses. *Frontiers in Plant Science*, 6, 600. Available from: https://doi.org/10.3389/fpls.2015.00600

Zeng, H., Zhang, Y., Zhang, X., Pi, E. & Zhu, Y. (2017) Analysis of EF-hand proteins in soybean genome suggests their potential roles in environmental and nutritional stress signaling. *Frontiers in Plant Science*, 8, 877. Available from: https://doi.org/10.3389/fpls.2017.00877

Zhang, J., Zou, A., Wen, Y., Wei, X., Liu, C., Lv, X. et al. (2022) SlCML55, a novel *Solanum lycopersicum* calmodulin-like gene, negatively regulates plant immunity to Phytophthora pathogens. *Scientia Horticulturae*, 299, 111049. Available from: https://doi.org/10.1016/j.scienta.2022.111049

Zhang, L., Du, L., Shen, C., Yang, Y. & Poovaiah, B.W. (2014) Regulation of plant immunity through ubiquitin-mediated modulation of Ca(2+) -calmodulin-AtSR1/CAMTA3 signaling. *The Plant Journal*, 78(2), 269–281. Available from: https://doi.org/10.1111/tpj.12473

Zhang, Y., Xu, S., Ding, P., Wang, D., Cheng, Y.T. & He, J. et al. (2010) Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. *Proceedings of the National Academy of Sciences of the United States of America*, 107(42), 18220–18225. Available from: https://doi.org/10.1073/pnas.1005225107

Zhao, C., Tang, Y., Wang, J., Zeng, Y., Sun, H., Zheng, Z. et al. (2021) A mis-regulated cyclic nucleotide-gated channel mediates cytosolic calcium elevation and activates immunity in Arabidopsis. *New Phytologist*, 230(3), 1078–1094. Available from: https://doi.org/10.1111/nph.17218

Zhao, Y., Liu, W., Xu, Y.P., Cao, J.Y., Braam, J. & Cai, X.Z. (2013) Genome-wide identification and functional analyses of calmodulin genes in Solanaceous species. *BMC Plant Biology*, 13(70), 70. Available from: https://doi.org/10.1186/1471-2229-13-70

Zhou, J.M. & Zhang, Y. (2020) Plant immunity: danger perception and signaling. *Cell*, 181(5), 978–989. Available from: https://doi.org/10.1016/j.cell.2020.04.028

Zhu, X., Caplan, J., Mamillapalli, P., Czymmek, K. & Dinesh-Kumar, S.P. (2010) Function of endoplasmic reticulum calcium ATPase in innate immunity-mediated programmed cell death. *The EMBO Journal*, 29(5), 1007–1018. Available from: https://doi.org/10.1038/emboj.2009.402

Zhu, X., Dunand, C., Snedden, W. & Galaud, J.P. (2015) CaM and CML emergence in the green lineage. *Trends in Plant Science*, 20(8), 483–489. Available from: https://doi.org/10.1016/j.tplants.2015.05.010

Zhu, X., Robe, E., Jomat, L., Aldon, D., Mazars, C. & Galaud, J.P. (2016) CML8, an Arabidopsis calmodulin-like protein, plays a role in *Pseudomonas syringae* plant immunity. *Plant and Cell Physiology*, 58(2), pcw189. Available from: https://doi.org/10.1093/pcp/pcw189

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J.D.G., Boller, T. et al. (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. *Cell*, 125(4), 749–760. Available from: https://doi.org/10.1016/j.cell.2006.03.037

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