Ca\textsuperscript{2+}/Calmodulin Complex Triggers CAMTA Transcriptional Machinery Under Stress in Plants: Signaling Cascade and Molecular Regulation

Zahra Iqbal\textsuperscript{1}, Mohammed Shariq Iqbal\textsuperscript{2}, Surendra Pratap Singh\textsuperscript{3} and Teerapong Buaboocha\textsuperscript{1,4}\textsuperscript{*}

\textsuperscript{1} Molecular Crop Research Unit, Department of Biochemistry, Chulalongkorn University, Bangkok, Thailand, \textsuperscript{2} Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Lucknow Campus, Lucknow, India, \textsuperscript{3} Plant Molecular Biology Laboratory, Department of Botany, Dayanand Anglo-Vedic (PG) College, Chhatrapati Shahu Ji Maharaj University, Kanpur, India, \textsuperscript{4} Omics Sciences and Bioinformatics Center, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

Calcium (Ca\textsuperscript{2+}) ion is a critical ubiquitous intracellular second messenger, acting as a lead currency for several distinct signal transduction pathways. Transient perturbations in free cytosolic Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{cyt}) concentrations are indispensable for the translation of signals into adaptive biological responses. The transient increase in [Ca\textsuperscript{2+}]\textsubscript{cyt} levels is sensed by an array of Ca\textsuperscript{2+} sensor relay proteins such as calmodulin (CaM), eventually leading to conformational changes and activation of CaM. CaM, in a Ca\textsuperscript{2+}-dependent manner, regulates several transcription factors (TFs) that are implicated in various molecular, physiological, and biochemical functions in cells. CAMTA (calmodulin-binding transcription activator) is one such member of the Ca\textsuperscript{2+}-loaded CaM-dependent family of TFs. The present review focuses on Ca\textsuperscript{2+} as a second messenger, its interaction with CaM, and Ca\textsuperscript{2+}/CaM-mediated CAMTA transcriptional regulation in plants. The review recapitulates the molecular and physiological functions of CAMTA in model plants and various crops, confirming its probable involvement in stress signaling pathways and overall plant development. Studying Ca\textsuperscript{2+}/CaM-mediated CAMTA TF will help in answering key questions concerning signaling cascades and molecular regulation under stress conditions and plant growth, thus improving our knowledge for crop improvement.

Keywords: calcium, calmodulin, development, transcription factor, CAMTA, stress

INTRODUCTION

Plants have evolved several strategies, majorly through molecular mechanisms, to optimize growth and ameliorate tolerance toward environmental constraints (Tripathi et al., 2020). One of the basic mechanisms is an increase in the concentration of [Ca\textsuperscript{2+}]\textsubscript{cyt} in response to various external stimulus (Hepler, 2005). Ca\textsuperscript{2+} signatures are considered as core regulators of many adaptive and developmental processes. They are characterized by stimulus-driven signals resulting from the cumulative action of carriers, pumps, and channels. These transporters drive Ca\textsuperscript{2+} signals spatially and temporally to act inside a cell. Spatial and temporal Ca\textsuperscript{2+} signals are transmitted and decoded by a bunch of Ca\textsuperscript{2+}-binding proteins such as calmodulin (CaM) and calcineurin. These proteins further spread relayed information to generate specific downstream response by regulating transcription factors (TFs). TFs are DNA-binding regulatory proteins involved in distinctive expression of genes that regulate developmental processes and environmental stress responses.
(Hong, 2016; Mitsis et al., 2020). CaM, upon interaction with Ca\(^{2+}\), undergoes conformational changes to modulate several TFs (Reddy, 2001). TFs contribute to various facets of cellular processes and act as a toolkit to signals perceived from within and outside an organism (Mitsis et al., 2020). Intriguingly, TFs constitute a major portion of eukaryotic genome, accounting for approximately 5% of the total genome (approximately 2,000 genes) in humans (Tupler et al., 2001). In Arabidopsis thaliana, 11.8% of their genome encodes for TFs (approximately 3,000 genes) (Arabidopsis Genome Initiative, 2000). TFs such as DREB (Agarwal et al., 2006; Chen et al., 2008; Lata and Prasad, 2011; Jan et al., 2017), NAC (Tran et al., 2004; Nakashima et al., 2007; Puranik et al., 2012; Marques et al., 2017), bHLH (Sun et al., 2018; Wang et al., 2020), WRKY (Park et al., 2005; Phukan et al., 2016), and MYB (Levy et al., 2005; Roy, 2016; Zhang et al., 2018) had been previously reported to play crucial roles in stress biology and plant growth. In a similar vein, CAMTA (calmodulin-binding transcription activator) in a Ca\(^{2+}\)/CaM-driven modus had been involved in carrying out important functions by modulating plant stress responses and overall development (Bouche et al., 2002, 2005; Galon et al., 2010a; Liu et al., 2015; Shkolnik et al., 2019). The present review recapitulates the progress made in comprehending the involvement of plant/Calmodulin-mediated CAMTA regulation in stress adaptation and plant development.

CALCIUM: A SECOND MESSENGER AND ITS INTERACTION WITH CALMODULIN

A second messenger is a molecule that acts to transmit signals from a receptor to a target (Bush, 1995). Ca\(^{2+}\) is one of the best characterized second messengers. It is hypothesized that Ca\(^{2+}\), as a messenger ion, emerged early during cell evolution (Edel et al., 2017). The role of Ca\(^{2+}\) as a primary nutrient in sustaining the structural integrity of cell walls and modulating diverse physiological processes such as biotic and abiotic stresses had been elucidated well by extensive biochemical and genetic studies (Poovaiah et al., 1993; Zielinski, 1998; Reddy, 2001; Snedden and Fromm, 2001; Sanders et al., 2002; Harper et al., 2004; Reddy et al., 2004; Bouche et al., 2005; Hepler, 2005; Aghdam et al., 2012; Thor, 2019; Tian et al., 2020). Ca\(^{2+}\) spikes are usually the outcome of two opposing reactions occurring in cells: Ca\(^{2+}\) influx (entry) via dedicated channels or Ca\(^{2+}\) efflux (exit) via specific pumps (Xiong et al., 2006; Tuteja and Mahajan, 2007). The regulation of nanomolar concentration of Ca\(^{2+}\) is achieved by two mechanisms. First, Ca\(^{2+}\)-ATPases pump [Ca\(^{2+}\)]\(_{cyt}\) to the exterior or into organelles such as vacuoles and endoplasmic reticulum. Second, the opening of Ca\(^{2+}\)-permeable ion channels results in the Ca\(^{2+}\) influx to the cytosol (Medvedev, 2005; Tuteja and Mahajan, 2007; Swarbreck et al., 2013; Demidchik and Shabala, 2018). Transmembrane Ca\(^{2+}\) gradients maintain sufficient energy to drive Ca\(^{2+}\) flux passively into the cytosol from the apoplast or the organelles (Medvedev, 2005; Swarbreck et al., 2013; Demidchik and Shabala, 2018).

Much evidence indicates that Ca\(^{2+}\)-mediated signaling is implicated in the relay of stress signals such as light (Kim et al., 2003; Liang et al., 2009; Hochmal et al., 2015; Hou et al., 2019), temperature (Teige, 2019), salt (Rahman A. et al., 2016; Manishankar et al., 2018; Seifikalhor et al., 2019), cold (Shi et al., 2014; Yuan et al., 2018b), and gravity (Kordyum, 2003; Toyota et al., 2008; Salmi et al., 2011); oxidative signals such as pathogen attack (Kiep et al., 2015; Aldon et al., 2018; Tian et al., 2019) and reactive oxygen species (Mori and Schroeder, 2004; Monshausen et al., 2009; Kurusu et al., 2015); and hormone signals such as ethylene (Li et al., 2018; Zhu et al., 2018), abscisic acid (ABA) (Edel and Kudla, 2016; Chen et al., 2017; Yuenyong et al., 2018), gibberellins (Abbasi et al., 2004; Nakata et al., 2009; Li et al., 2013), and auxins (Vanneste and Friml, 2013; Hazak et al., 2019). In plants, oodles of Ca\(^{2+}\)-binding proteins function as Ca\(^{2+}\) sensors decoding complex Ca\(^{2+}\) signatures (Kudla et al., 2018). These binding proteins sense changes in [Ca\(^{2+}\)\(_{cyt}\)] and/or [Ca\(^{2+}\)\(_{nuc}\)], regulating downstream signaling processes and hence drawing physiological response against them (Day et al., 2002; Boonkrapong and Buaboocha, 2007). Ca\(^{2+}\) sensors are basically proteins with highly conserved one or multiple helix-turn-helix structures-EF-hand (Nakayama et al., 2000; Day et al., 2002). Approximately, 250 EF-hand comprising putative Ca\(^{2+}\) sensors are known in A. thaliana, which accounts for about 1% of the reported proteome (Day et al., 2002). Ca\(^{2+}\) sensors are divided into three categories, namely, (i) CaM and CMLs (calmodulin-like proteins) (Bender and Snedden, 2013), (ii) CBLs (calcineurin-B-like proteins) (Luan, 2009), and (iii) CPKs (Ca\(^{2+}\)-dependent protein kinases) and CCMK (calcium/calmodulin-dependent protein kinase) (Cheng et al., 2002).

Calmodulins are a class of extensively studied Ca\(^{2+}\) sensors and are known to regulate diverse cellular processes in plants, including stress responses and plant development (Zielinski, 1998; Bouche et al., 2002; Bergey et al., 2014; Zeng et al., 2015; Dubrovina et al., 2019). CaM is a 17-kDa dumbbell-shaped cytosolic acidic protein, with a supple joint in the middle (Meador et al., 1992). CaMs contain four EF-hand motifs and are highly conserved among eukaryotes (Zielinski, 1998; Reddy et al., 2002). It has been ascertained that around 300 proteins interact with CaMs in plants (Reddy et al., 2002; Bouche et al., 2005; Popescu et al., 2007). Among the known protein–protein interactions, CaMs are implicated to have maximum interacting partners (Lee et al., 2010). Conformational changes upon Ca\(^{2+}\)/CaM association lead to contact of hydrophobic surface within each domain (Liu et al., 2017). This ultimately elevates the CaMs Ca\(^{2+}\) sensor bustle, thus resulting in an interaction with its target proteins (Snedden and Fromm, 2001). CaM can act either directly by interacting with key target enzymes or indirectly with the help of specific kinases (Zielinski, 1998; Mt and Mn, 2008; Villalobo et al., 2019). Interestingly, Ca\(^{2+}\) ions interact with CaM in a cooperative manner, such that even small changes in the level of [Ca\(^{2+}\)\(_{cyt}\)] lead to huge alteration in the levels of active CaM (Beccia et al., 2015; Fernandes and Oliveira-Brett, 2017). Various CaM proteins display differential expression and possess variable affinity to Ca\(^{2+}\), including their downstream target proteins (McCormack et al., 2005; Popescu et al., 2007; Chinpongpanich et al., 2015). Many Ca\(^{2+}\) and Ca\(^{2+}\)/CaM-associated TFs, implicated in stress signaling, are found in plants (Singh and Virdi, 2013; Shen et al., 2020).
However, few TFs are specifically transcribed to distinct Ca\(^{2+}\) signal durations and amplitude. For example, the binding of Ca\(^{2+}\) can directly regulate the activity of certain TFs such as the DREAM (downstream regulatory element antagonist modulator) protein (Carrion et al., 1999). Similarly, AtNIG1 (\textit{A. thaliana} NaCl-inducible gene 1) is also classified as a Ca\(^{2+}\)-dependent TF, which is targeted in the nucleus, indicating that it is a nuclear Ca\(^{2+}\)-binding protein (Kim and Kim, 2006). Taking Ca\(^{2+}\)/CaM-associated TFs into account, AtGT2L, a member of GT-2 subfamily, upon interaction with Ca\(^{2+}\)/CaM, is responsive to freezing and salinity stresses in plants (Xi et al., 2012). WRKY is also implicated to be a Ca\(^{2+}\)/CaM-dependent gene (Park et al., 2005; Yan et al., 2018). AtWRKY7 is well reported to be involved in Ca\(^{2+}\)/CaM-dependent gene regulation and has a role in pathogen incursion (Park et al., 2005). Experimental results have revealed that CaM binds exclusively to the Ca\(^{2+}\)-regulated CaM-binding domain (CaMBD) of AtWRKY7 (Park et al., 2005). In addition, WRKY45, WRKY43, WRKY53, and WRKY50 bind to various isoforms of CaM in a Ca\(^{2+}\)-driven behavior (Popescu et al., 2007). Myb is another TF that is well characterized as a Ca\(^{2+}\)/CaM-dependent protein. It acts upstream to a number of defense-responsive, salinity, and drought-receptive genes (Do Heo et al., 1999; Stracke et al., 2001; Li et al., 2019). CaM also supports transcriptional repression interceded by CBNAC/NTL9 (Kim et al., 2007). The TF ZmNAC84 has been reported to interact physically with ZmCCaMK both \textit{in vivo} and \textit{in vitro}. ZmNAC84, after ABA induction, has a partially overlapping expression profile with ZmCCaMK. Its overexpression renders drought tolerance and resistance to oxidative stress induction owing to drought (Zhu et al., 2016). Moreover, Cam7, also known as ZBF3, is a CaM isoform in \textit{A. thaliana} that works as a transcriptional regulator. The upstream elements to TSS of light-inducible genes are directly targeted by ZBF3 to promote photomorphogenesis (Yadav et al., 2005; Kushwaha et al., 2008). In a similar context, recently, CaM proteins have also been implicated in salt stress. “Khao Dawk Mali 105” rice variety, which overexpressed OsCam1-1, shows differential expression of several genes implicated in salt stress signaling, hormonal regulation, lipid/carbohydrate/secondary metabolism, photosynthesis, etc. Owing to OsCam1-1 overexpression, the rate of photosynthesis declines in transgenic rice, whereas content of sucrose and starch increases under salt stress. This study further revealed that CaM under salt stress condition boosts several metabolic enzymes implicated in energy pathways of plant cells, which either conserve or generate energy under limited photosynthesis (Yuenyong et al., 2018). Thus, Ca\(^{2+}\)/CaM acts as an intermediate complex between stimulus perception and gene expression (Figure 1).

**CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR**

Calmodulin-binding transcription activator also known as SR (signal responsive) protein (Yang and Poovaiah, 2000) or EICBP (ethylene-induced CaM-binding proteins) (Reddy et al., 2000)
is considered as the largest and best characterized family of Calmodulin-binding TFs (Finkler et al., 2007; Kim et al., 2009). The presence of this novel protein that binds to DNA was for the very first time identified during the process of isolation of partial cDNA clone (CG-1) from parsley (Petroselinum crispum) (e Silva, 1994). It was shown that CGCG was the signature motif required for the binding of this protein to DNA. Upon exposure to UV-containing white light, the CG-1 coding mRNA was rapidly and transiently accumulated in parsley cultured cells; however, until then, CG-1 was not designated as a Ca\(^{2+}\)/Calmodulin-binding protein (e Silva, 1994). Later, Yang and Poovalia (2000) identified NtER1 as a Calmodulin-binding protein engaged in ethylene-regulated plant death and senescence, indicating its potential application in prolonged storage life of horticultural crops. They also showed that Calmodulin binds with a very high affinity (\(K_d\sim12\) nM) in a Ca\(^{2+}\)-dependent manner to NtER1 (Yang and Poovalia, 2000). Yang and Poovalia (2002) further reported a homolog of NtER1 and five related genes in Arabidopsis (AtSRs). Their results evidently suggested AtSRs as a Calmodulin-binding gene family with CGCG as a core motif in their promoters. The study also indicated probable implication of CAMTAs in numerous signal transduction pathways in plants (Yang and Poovalia, 2002). Since then, CAMTA TF had been reported in various multicellular organisms. With the advancement in computational techniques, proteins similar to CAMTA had been reported in unicellular eukaryotes such as the ciliates, Paramaecium tetraurelia and Tetrahymena thermophila (Finkler et al., 2007). The orthologs of CAMTA had also been reported in Arabidopsis (Yang and Poovalia, 2002; Bouche et al., 2005); humans (Song et al., 2006); flies (Han et al., 2006); and in various crop plants such as tobacco (Yang and Poovalia, 2000), rice (Choi et al., 2005), tomato (Yang et al., 2012), grapevine (Shangguan et al., 2014), corn (Yue et al., 2015), strawberry (Leng et al., 2015), barrelclover (Yang et al., 2015), soybean (Wang et al., 2015), poplar (Wei et al., 2017), and cotton (Pant et al., 2018). Deciphering the CAMTA gene expression patterns and functional redundancies in model plants and various crops laid a foundation for further investigation of its role, which can be beneficially applied to agricultural sector. CAMTA as a TF has been extensively studied for its involvement in stress and developmental biology (Galon et al., 2008; Doherty et al., 2009; Pandey et al., 2013; Sun et al., 2020) (discussed in CAMTA Transcriptional Response Under Abiotic Stress Conditions and CAMTA Transcriptional Response Under Biotic Stress Conditions).

The most important characteristic of this TF is its affinity for Calmodulin, indicating its participation in Ca\(^{2+}\) sensing. Calmodulin signatures are effectively decoded by CAMTAs to generate explicit gene expression patterns (Liu et al., 2015). Liu et al. deciphered how different Ca\(^{2+}\) signatures are decoded by CAMTAs for specific gene expression patterns. The group devised a dynamic model based on thermodynamic and kinetic principles for understanding the Ca\(^{2+}\)-Calmodulin–CAMTA binding and the subsequent gene expression patterns. The modeling analysis unraveled that Ca\(^{2+}\) signals in the form of elevated \([\text{Ca}^{2+}]_{\text{c}}\) are non-linearly amplified upon binding of Ca\(^{2+}\), Calmodulin, and CAMTAs. They further combined experimental data with mathematical modeling to comprehend the information flow from Ca\(^{2+}\) signatures to CAMTAs—regulated gene expression patterns (Liu et al., 2015). This study provided great insight about the involvement of Ca\(^{2+}\)/Calmodulin in regulation of CAMTAs. The succeeding section focuses on the transcriptional machinery of CAMTA with respect to its molecular and physiological functions in regulating stress responses and overall plant development.

### CAMTA Domain Organization

Calmodulin-binding transcription activators were reported to consist of multiple functional domains (Finkler et al., 2007). These domains have evolutionary conserved amino acid sequence, organized into a specific conserved order. The predicted functional domains of CAMTA include (a) NLS (nuclear localization signals) for targeting protein into nucleus. These signals have been reported in almost all the CAMTAs, but their localization varies in different organisms; (b) CG-1 domain, which is a unique domain implicated in DNA binding; for any protein to be characterized as CAMTA, the presence of CG-1 domain is obligatory; (c) TIG, which is involved in non-specific DNA interactions in TFs (Aravind and Kooshin, 1999) and also implicated in protein dimerization (Muller et al., 1995); (d) ankyrin (ANK) repeats, which are tandem repeats of about 33 amino acids in a variety of eukaryotic and viral proteins and contribute in protein–protein interactions (Sedgwick and Smerdon, 1999; Rubtsof and Lopina, 2000); (e) CaMBD, which is implicated in the association of Ca\(^{2+}\)-loaded Calmodulin to CAMTAs; (f) IQ motifs, which are implicated in the binding of Calmodulin and Calmodulin-like proteins and are regions of low complexity with a consensus motif IQXXXRGGXXX (Bahler and Rhoads, 2002). In some cases, the presence of transcription activation domains (TADs) has also been mapped. The presence of TAD was reported in AtCAMTA1 (Bouche et al., 2002), HsCAMTA2 (Song et al., 2006), and DmCAMTA (Han et al., 2006). However, owing to lack of sequence homology in this domain, it cannot be inferred that all CAMTAs possess TAD. All these domains are pivotal for the transcription of CAMTA. Nonetheless, certain variations exist; for example, the mapping of Ca\(^{2+}\)-loaded Calmodulin domain on AtCAMTA1 reveals a binding site adjacent to IQ motif, indicating multiple Calmodulin binding sites (Bouche et al., 2002). Similarly, in OsCAMTA, the Ca\(^{2+}\)-dependent Calmodulin and Ca\(^{2+}\)-independent Calmodulin dissociation domains were mapped on C-termini (Choi et al., 2005). Recently, in Gossypium species, GrCAMTA5.2, GrCAMTA5.3, and GhCAMTA3D.1, GhCAMTA5D.1 were depicted as TIG lacking CAMTAs in Gossypium raimondii and Gossypium hirsutum, respectively (Pant et al., 2018). Similar to Gossypium study, five TaCAMTAs (Triticum aestivum CAMTA), namely, TaCAMTA1-A/B/D and TaCAMTA5-A/D, also lacked TIG domain (Yang et al., 2020). The domain organization of six Arabidopsis CAMTAs, analyzed bioinformatically, is shown in Figure 2.

### CAMTA Transcriptional Response Under Abiotic Stress Conditions

The underpinning mechanisms of CAMTA under abiotic stress conditions had been reasonably elucidated (Pandey et al., 2013; Noman et al., 2019; Shkolnik et al., 2019). Under
normal conditions, where plants are not exposed to any stress, there is minimal induction of CAMTA genes. This could be either because the genes in this family of TFs share functional redundancy or the genes in this family are expressed under certain specific environmental conditions. Taking the model plant *A. thaliana* into account, six CAMTAs were reported: CAMTA1, CAMTA2, CAMTA3, CAMTA4, CAMTA5, and CAMTA6. It was shown that all these genes were rapidly and differentially triggered by environmental cues such as high temperatures, salinity, H$_2$O$_2$, physical wounds; and hormones such as ethylene, ABA, methyl jasmonate (MeJA), and salicylic acid (SA) (Yang and Poovaiah, 2002; Kim et al., 2013, 2017; Pandey et al., 2013; Shkolnik et al., 2019). Considering the first member of *Arabidopsis* CAMTA gene family, AtCAMTA1, its role has been reported in auxin signaling (increased expression of AUX/IAA-IAA29), transport, and homeostasis. For transgenic plants possessing AtCAMTA1 promoter, GUS construct displayed cell-specific expression profiles of auxin. The involvement of CAMTA1 in auxin signaling is established by chemical impairment of polar auxin transport. Gene expression profiling of auxin transport inhibitor 1-N-naphthylphthalamic acid to plants also supports the responsiveness of CAMTA1 in auxin pathways. Genome-wide transcriptome of *camta1* mutant mapped 63 upregulated genes. Further analysis revealed that 17 genes were involved in auxin signaling. Moreover, on analysis of hypocotyl elongation, the *camta1* mutants were hyperresponsive to auxin exposure as compared to wild type (Galon et al., 2010a). It was further reported that CAMTA1, CAMTA2, and CAMTA3 are negative regulators of auxin and are associated with genes responsible for red light and high light responses, whereas CAMTA4, CAMTA5, and CAMTA6 are positive regulators of auxins and are associated with genes responsible for blue light and darkness responses (Galon et al., 2010b). In a similar vein, phytohormonal regulation of CAMTAs is also extended to brassinosteroid (BR) signaling in which BZR1 (involved in BR signaling cascade) has CAMTA5 as an associated protein (Wang et al., 2013).

Additionally, *camta1* responded severely to cold stress, indicating its role in cold stress management. CAMTA protein provides a much-needed connecting link between Ca$^{2+}$ signatures and cold acclimatization. CBF TF plays a pivotal role in cold acclimation of plants. In *A. thaliana*, CBF genes induce approximately 100 more genes (termed as CBF regulon) upon exposure to low temperatures (Maruyama et al., 2004; Vogel et al., 2005). CAMTA binds to consensus sequence located in the promoter of the CBF2 gene. The 1,000-bp upstream region of CBF2 gene comprises seven conserved consensus motifs: CM1 to CM7. The CAMTA signature domain CG-1
matches the CM2 motif of CBF2. CBF2 expression is impaired in camta3 mutants and impaired to a much higher extent in camta1 and camta3 double mutant. These are indications of the intersecting functions of different CAMTA proteins. Therefore, CAMTA controls CBF regulon, which confers freezing tolerance to plants (Doherty et al., 2009). Moreover, CAMTA1, CAMTA2, and CAMTA3 work in recital to induce CBF1, CBF2, and CBF3 and confer freezing tolerance to plants at low temperatures. Furthermore, at warmer temperatures, these three CAMTAs collectively inhibit SA biosynthesis. It had been previously shown that exposure to low temperatures increases SA levels (Scott et al., 2004). SA biosynthesis at low temperatures involves ICS (inositol synthase) pathway, encircling ICS1, CBP60g, and SARD1 genes. At warm temperatures, CAMTA1, CAMTA2, and CAMTA3 suppress the accretion of ICS1, CBP60g, and SARD1. Such suppression by CAMTA proteins was not observed at low temperatures (Kim et al., 2013). More neoteric inventions have elucidated that the suppression of genes involved in SA biosynthesis by CAMTA at high temperatures (22°C) is overcome when plants are infected by biotrophic or hemibiotrophic pathogens and grown at low temperatures (4°C) for more than a week. Suppression of SA pathway by CAMTA3 under normal condition involves an N-terminal repression module (NRM). NRM works autonomously of CaM binding to CaMBD or the IQ. This finding is different owing to the fact that CAMTA3 repressional assertion involves the binding of CaM to CaMBD. To sum up, the repression activity of CAMTA3 at low temperatures and pathogen infection is governed by related mechanisms (Kim et al., 2017). To further elaborate, three dehydration-responsive element (DRE) binding protein 1/C-repeat binding factors (DREB1/CFBs) are master regulators of cold-responsive gene expression. DREB1 stimulates cold-responsive transcriptional cascade, eventually inducing several genes implicated in cold stress response. Rapid and gradual temperature decreases are sensed as cold stress by plants, which results in the activation of DREB1 genes. CAMTA3 and CAMTA5 are responsive to rapid decline in temperatures by inducing the expression of DREB1s. However, CAMTA3 and CAMTA5 do not respond to gradual declines in temperatures. In contrast to circadian clock associated 1 and late elongated hypocotyl (acts as transcriptional activators to regulate DREB1 expression only during the day), AtCAMTA3 and AtCAMTA5 function both during the day and night (Kidokoro et al., 2017).

Drought and salinity are considered as few of the worst conditions in agriculture and horticulture. The role of CAMTA1 has been extended to drought response (Pandey et al., 2013). The mutants had impaired photosystem II efficiency and water use efficiency, hence making plants susceptible to drought. The mutants had relatively low water content and retarded growth. The mutants had impaired photosystem II efficiency and water use efficiency, hence making plants susceptible to drought. Microarray analysis revealed drought revival, osmotic balance, apoptosis, DNA methylation, and photosynthesis as investigative pathways in drought-treated camta1 mutants. It has also been shown that CAMTA1 regulates a broad spectrum of stress-inducible genes such as RD26, ERD7, RAB18, LTPs, COR78, CBF1, and HSPs. To ascertain the claims, CAMTA recognition cis-element was found to be enriched in the 1,000-bp upstream regions of the listed genes (Pandey et al., 2013). Very recently, CAMTA6 has been shown to be associated with sodium (Na+) homeostasis during early seed germination. The camta6 mutants accumulate less NaCl and display tolerance to salinity and ABA. AtHKT1 (high affinity K+ transporter 1 encoding a Na+/K+ transporter) expression was limited to radicles and did not increase upon subjecting camta6 to salt stress or ABA treatment. In addition, even the transcriptome of CAMTA6 under control and salt stress condition revealed 1,020 upregulated and 1,467 downregulated salt-responsive genes in the wild type (Shkolnik et al., 2019). With regard to context, although CAMTA2 in plants has been less explored, studies highlight the regulation of AtALMT1 (aluminum-activated malate transporter 1) by CAMTA2 in association with WRKY46 (Tokizawa et al., 2015; Wu et al., 2019). Next, considering the involvement of CAMTAs in plant development, the role of CAMTA1 and CAMTA5 is also extended in pollen development. The AVP1 promoter activity has been reported to be activated by AtCAMTA1 in cultured cells. AtCAMTA1 triggers the GUS reporter expression driven by P281 promoters of AVP1 but does not stimulate the GUS reporter expression in mutants with disrupted despared CGCG-box, indicating that AtCAMTA1 attaches to CGCG-box and elevates AVP1 expression. AtCAMTA1 in collaboration with AtCAMTA5, cooperatively elevates their gene function and enhance AVP1 expression during pollen development (Mitsuda et al., 2003). Apart from A. thaliana, CAMTAs from other plant species have also been implicated very well in abiotic stress responses. Various in silico and quantitative reverse transcription–polymerase chain reaction (qRT-PCR) approaches were deployed to assess the involvement of CAMTAs under different abiotic stress conditions. For example, Zea mays (corn) ZmCAMTAs in drought, cold, salt, and hormonal signaling (Yue et al., 2015); Fragaria ananassa (Strawberry) FcCAMTAs in heat, cold, salt, and ethylene stress (Leng et al., 2015); T. aestivum (wheat) TaCAMTAs in cold, salt, heat, and drought stress (Yang et al., 2020); Citrus sinensis (sweet orange) CcCAMTAs in salinity, dehydration, and hormonal response (Zhang et al., 2019); and Linum usitatissimum (Flax) LuCAMTAs in drought, low temperature, and light responses (Ali et al., 2020) (discussed in detail in Recent Discoveries of CAMTAs in Various Crop Plants).

**CAMTA Transcriptional Response Under Biotic Stress Conditions**

Transient Ca2+ levels are known to be elevated during pathogen attack (Ma and Berkowitz, 2007), which eventually activates CaM. Similar to the role of CAMTA1 in abiotic stress tolerance, the role of CAMTA3 in response to biotic stress has been elucidated to a great extent (Galon et al., 2008; Lakul et al., 2012; Benn et al., 2016; Kim et al., 2017, 2020; Jacob et al., 2018). Ca2+-loaded CaM regulates the activity of CAMTA3 either by negative regulation of CAMTA3 or by positive regulation of a negative repressor TF (Galon et al., 2008). The bacterial pathogen Pseudomonas syringae and the fungal pathogen Botrytis cinerea did not heavily affect camta3 in comparison to their effect in control plants. This is suggestive of the fact that CAMTA3 is responsible for suppression of biotic defense responses, which may be primarily achieved either by binding of CAMTA3 with...
the promoters of suppressed genes or by expressing a TF that is repressed. Transcriptomics approach on camta3 revealed an attenuated expression of six genes and an enhanced expression of 99 genes out of which 32 genes are related to defense response such as WRKY33, PR1, chitinase, etc. Apart from model plants, the role of CAMTA3 in biotic response was also extended to crop plants. OsCBT encoding CaM-binding protein was functionally characterized in plant defense. Oscht-1 mutants were significantly tolerant to rice blast fungus Magnaporthe grisea and the bacterial pathogen Xanthomonas oryzae (Choi et al., 2005; Koo et al., 2009). Furthermore, for the identification of transcriptional network governed by OsCBT, transcriptome analysis between wild-type (WT) and oscht-1 rice grown in pathogen-free conditions was performed. The analysis revealed 81 up-regulated genes and 200 down-regulated genes. Majority of these genes were implicated in biotic stress responses. The gene expression patterns of fungal pathogen response were also found to be significantly affected in oscht-1 mutant, suggesting the probability of OsCBT in regulating rice defense response (Chung et al., 2020).

Pathogen attack on plants causes intracellular Ca$^{2+}$ spikes; however, how Ca$^{2+}$ mediates this interaction-based SA level fluctuation remains unclear. CAMTA3 has been reported to link Ca$^{2+}$ transients to SA-mediated immune response. CAMTA3 also interacts with EDS1 promoter to impair its expression (Du et al., 2009). Further research has led to the establishment of the role of CAMTA3 in plant defense and ethylene-induced senescence (Nie et al., 2012). Further research has led to the establishment of the role of CAMTA3 in plant defense and ethylene-induced senescence (Nie et al., 2012). Edr2 (enhanced disease resistance 2) suppressors were screened to establish the components of EDR2 pathways. This led to the identification of gain-of-function mutation in signal responsive1 (SRI) or CAMTA3. Edr2 mutants have enhanced tolerance for Golovinomyces cichoracearum (powdery mildew), indicating that EDR2 is a negative regulator for powdery mildew. The srl-1D mutation restrains edr2-mediated powdery mildew tolerance. Post mildew infection, as compared to wild type, the edr2 plants displayed necrotic lesions with little powder. However, the edr2 srl-1D plants phenotypically resemble that of the control plants with oodles of conidia on leaves. The srl-1D gain-of-function also represses elevated ethylene induced senescence. Thus, CAMTA3 negatively impacts plant immune responses. It also directly interacts with the promoter of NDR1 (non-race specific disease resistance1), a key module in resistance to P. syringae-mediated plant immunity. Moreover, CAMTA3 also regulates EIN3 (ethylene insensitive3), which contributes to ethylene-induced senescence (Nie et al., 2012).

As discussed previously, CAMTA1, 2, and 3 play pivotal roles in SA-mediated plant immunity. Pip (piperoc acid) regulates the process of systemic acquired resistance (SAR) in plants (Bernsdorff et al., 2016). CAMTA1, 2, and 3 suppress the synthesis of Pip by inducing ALD1 (AGD2-like defense response protein 1). ALD1 is known to encode an enzyme required for Pip biosynthesis. The induction of ALD1 results in an accumulation of Pip, which in turn increases the concentration of NPR1 (SA receptor protein). Thus, CAMTA123 triple mutation induces plant defense machinery and instigates SAR (Kim et al., 2020). Moreover, CBP60g and SARD1 TFs are regulators of SA and NHP (N-hydroxypipecolic acid). Both SA and NHP have been extensively linked to plant immunity. CBP60g has been identified as a direct target of CAMTA3 through chromatin immuno-precipitation (ChIP) and electrophoretic mobility shift assay (EMSA) assays. Thus, CAMTA3 suppresses SA and NHP biosynthesis by regulating SARD1 and CBP60g expression (Lenzoni et al., 2018; Sun et al., 2020).

CAMTA3, as defense against insect herbivory, has also been studied in plants (Qiu et al., 2012). Loss-of-function mutation of AtSR1 suppresses resistance to feeding by Trichoplusia ni (generalist herbivore), sustaining radically elevated larval weight gains and jasmonate (JA) accumulation owing to wound. The responsiveness of AtSR1 mutant to T. ni is attributed to low glucosinolate (GS) levels with remarkably decreased levels in indol-3-ylmethyl (13M) and 4-methylsulfinylbutyl (4MSOB), the two well-characterized herbivory deterrents. Induction of the various genes implicated in GS metabolism such as IQR1, MYB51, and AtST5a also changes with alterations in AtSR1 transcript levels. Thus, AtSR1 through Ca$^{2+}$ signaling cascade acts as a key module of plant resistance to insect herbivory. Additionally, Ca$^{2+}$/CaM-dependent signaling plays a major role in GS metabolism through CAMTA3 involvement (Laluk et al., 2012). Furthermore, upon pathogen exposure, plants allocate their energy resources (at the expense of growth) to defend themselves against invading pathogens. Yuan et al. (2018a) well-documented AtSR1 as a negative regulator of plant immune responses (PTI-, ETI-, SA-, and JA-mediated signaling pathways) and a positive regulator of plant growth (auxin and BR signaling pathways). EMSA and ChIP assays demonstrated that AtSR1 maintains a strong balance between plant immunity and growth by interacting with the CGCG motif present in the upstream sequences of its potential target genes (Yuan et al., 2018a). The same has also been previously established by Li et al. (2014), who identified seven SISR/CAMTA genes in tomato (Solanum lycopersicum). Functional analysis using VIGS indicated that both SISR1 and SISR3L negatively regulate plant defense responses. SISRs were explicitly induced upon P. syringae pv. tomato (Pst) DC3000 and B. cinerea infection. The knockouts of SISR1 or SISR3L displayed higher tolerance to Pst DC3000 and B. cinerea with subsequent accumulation of H$_2$O$_2$. Moreover, the genes implicated in pattern-triggered immunity, defense responses, and ethylene and SA pathways were significantly elevated (Li et al., 2014).

To cope with environmental constraints, plants have synchronized mechanisms underlying GSR (general stress response) networks and stress-specific networks (Benn et al., 2014). RSRE (rapid stress response element), the functional motif of GSR, has been extensively studied to explore the new horizons of stress signaling (Walley and Dehes, 2010). CAMTA3 has been very well studied as a pivotal modulator of RSRE-mediated GSR (Bjornson et al., 2014). The function of CAMTA in RSRE response was hypothesized on the basis that Ca$^{2+}$ ion inducers, flagellin22 (flg22) and oligogalacturonic acid, enhance the RSRE activity, and Ca$^{2+}$ ion chelator, EGTA, reduces the RSRE activity upon wounding. CAMTA3 lies downstream of MEEK1, facilitating the regulation of peak time and amplitude of plant GSR and, in conjugation with CAMTA2 and CAMTA4,
Recent Discoveries of CAMTAs in Various Crop Plants

The function of CAMTAs has been established in crop plants like corn (Z. mays), strawberry (F. ananassa), barley (Hordeum vulgare), poplar (Populus trichocarpa), soybean (Glycine max), grapevine (Vitis vinifera), tomato (S. lycopersicum), and cotton (Gossypium spp.), where they were previously and largely unknown (Yang et al., 2013, 2015; Shangguan et al., 2014; Leng et al., 2015; Wang et al., 2015; Yue et al., 2015; Wei et al., 2017; Pant et al., 2018). Nine ZmCAMTAs have been reported, upon scanning the entire genome of maize. These CAMTAs were shown to share homology with rice CAMTA. Moreover, an exceedingly high degree of sequence and structural pattern existed among the ZmCAMTAs, indicating that their origin is from a single ancestral sequence. All these CAMTAs possess highly tissue-specific expression and display slight diversity in their gene organization. The qRT-PCR of ZmCAMTAs under various abiotic stress (drought, salt, and cold), biotic stress (RBSV-rice black-streaked dwarf virus infection), and stress hormones (ABA, auxin, SA, and MeJA) reveals its role as a stress-responsive gene. The occurrence of many stress-related cis-regulatory element in the 1.5-kb promoter region of ZmCAMTAs further confirmed its role in stress regulation (Yue et al., 2015). Taking into account tomato (S. lycopersicum), a horticulturally important crop, signal-responsive (SR)/CAMTA TF is well implicated in postharvest biology. SISR, in response to stress conditions, also coordinate Ca^{2+} signaling with signal transduction pathways implicated in stress during fruit ripening and storage, thus indicating its significant role in horticultural sector (Yang et al., 2013). As previously discussed, SISR are involved in plant defense responses and drought management. In S. lycopersicum, SISR1 and SISR3L negatively regulate plant defenses against B. cinerea and Pst DC3000. Conversely, SISR1L is involved in the positive regulation of drought stress responses (Li et al., 2014).

In F. ananassa, 4 CAMTAs, namely, FaCAMTA1, FaCAMTA3, FaCAMTA4, and FaCAMTA5, have been reported, upon the exploitation of BLAST and HMMER tool bioinformatically, using the CAMTA family domain file. All these CAMTAs possessed known domain organization comprising CG1, TIG, ANK repeats, and IQ motifs. Under heat, cold, salt, and ethylene stresses, the FaCAMTAs exhibit distinct expression patterns (Leng et al., 2015). CAMTA genes have also been identified in M. truncatula genome, specifying its role in nodule organogenesis. The MtCAMTA genes display tissue-specific expression and respond to various stress-related hormones. Upon Sinorhizobium meliloti infection, the expression profiling of the MtCAMTA genes revealed that there is an alleviation in the suppression of most MtCAMTA genes expression, indicating the CAMTA involvement in early nodulation signaling pathway. Moreover, the promoters of early rhizobial infection response genes display the presence of CAMTA binding motifs (Yang et al., 2015). Similarly, seven CAMTAs were identified in the genome of P. trichocarpa, and their expression patterns were analyzed in roots and leaves. The qRT-PCR analysis showed that their expression was induced by pathogenic infection with Alternaria alternata; mannitol, NaCl, and cold stress; and phytohormones including SA, ABA, and MeJA (Wei et al., 2017). This was further substantiated as their promoter analysis revealed that most PpCAMTAs contain phytohormone or stress-related cis-regulatory elements. In G. max, 15 CAMTA genes were identified, sharing sequence homology with their Arabidopsis counterparts. All GmCAMTAs were profoundly expressed in root and leaf tissue. Their transcript abundance was induced significantly upon dehydration, cold, salinity, and hormonal treatments such as ABA, SA, and MeJA. In concurrence to their receptiveness to these signals, the promoter regions of these genes have been reported to be enriched with stress-related cis-regulatory elements (Wang et al., 2015). Complete functional analysis of GmCAMTA12, in relation to drought stress, has been performed. The promoter region of GmCAMTA contained ABRE, SARE, G-box, and W-box cis-regulatory elements. Arabidopsis T3 overexpression lines of GmCAMTA12 display enhanced drought tolerance and increased survival and germination rate under drought. GmCAMTA12 overexpression lines performed better in terms of physiological parameters such as proline and malondialdehyde contents, catalase activity, and electrolyte leakage (Noman et al., 2019). In V. vinifera, 10 SR/CAMTAs were identified bioinformatically as Ca^{2+}/CaM-binding protein, belonging to four gene groups: VcCAMTA1, VcCAMTA3, VcCAMTA4, and VcCAMTA5 and localized on 5, 7, 1, and 5 chromosomes, respectively. Expression analysis of VcCAMTAs depicted its role mainly in Ca^{2+} signal relay, with significantly high expression in bud, fruit, and inflorescence (Shangguan et al., 2014). Additionally, in four different Nicotiana species (Nicotiana tabacum, Nicotiana sylvestris, Nicotiana tomentosiformis, and Nicotiana benthamiana), 29 CAMTAs (13 in N. tabacum, 6 in N. sylvestris, and 5 each in N. tomentosiformis
and *N. benthamiana* were identified by evolutionary and expression analyses, which provided great insights into CAMTAs origin, expansion, and response toward stress conditions and plant development (Kakar et al., 2018). *NtABCAMTAs* are among the early responsive genes to biotic stress (*cucumber mosaic virus*-M-M strain of CMV, *potato virus Y*-Mn strain of PVY, and fungal pathogen black shank or *Phytophthora nicotianae*) and played pivotal roles in plant defense. *NtABCAMTAs* were also significantly responsive to abiotic conditions such as cold, drought, and cadmium stress (Kakar et al., 2018).

The identification and functional characterization of CAMTA gene family were also extended to cotton. Six CAMTA members were identified in *Gossypium arboreum*, 7 in *G. raimondii*, and 9 in *G. hirsutum*. Segmental duplication and purifying selection play key roles in the expansion and evolution of CAMTAs in cotton genome. Expression profiling indicated that cotton CAMTAs were predominantly expressed in various stages of fiber development (0, 6, 9 12, and 25 days after anthesis). Precisely, the specific involvement of *GhCAMTA2A.2* and *GhCAMTA7A* in maintaining fiber strength has been reported.
**TABLE 1** | Reported functions of CAMTA in plants.

| Gene name      | Function                                                                 | Source organism                        | Reference                               |
|----------------|--------------------------------------------------------------------------|----------------------------------------|-----------------------------------------|
| *AtCAMTA1*     | Drought tolerance via ABA signaling                                       | *Arabidopsis thaliana*                 | Pandey et al., 2013                      |
|                | Cold acclimatization                                                      | *A. thaliana*                          | Doherty et al., 2009                     |
|                | Repressor of ICS1, CBP60g, and SARD1 (suppressor of SA accumulation)     | *A. thaliana*                          | Kim et al., 2013; Sun et al., 2020       |
|                | Salt stress response                                                       | *A. thaliana*                          | Galon et al., 2010a                      |
|                | Auxin homeostasis, transport, and signaling                              | *A. thaliana*                          | Galon et al., 2010a,b                    |
| *AtCAMTA2*     | Suppressor of SA biosynthesis-related gene transcripts                    | *A. thaliana*                          | Kim et al., 2013; Sun et al., 2020       |
|                | Activator of AtALMT1 (metal toxicity)                                    | *A. thaliana*                          | Tokizawa et al., 2015; Wu et al., 2019   |
| *AtCAMTA3*     | Actuator of RSRE                                                           | *A. thaliana*/*Nicotiana benthamiana*  | Benn et al., 2014                        |
|                | Positive regulator of CBF2                                                | *A. thaliana*                          | Doherty et al., 2009                     |
|                | Suppressor of SA biosynthesis                                             | *A. thaliana*                          | Kim et al., 2013; Sun et al., 2020       |
|                | Suppressor of plant defense responses                                     | *A. thaliana*                          | Galon et al., 2008; Du et al., 2009      |
| *AtCAMTA4*     | Plant defenses against insect herbivory via SA-JA crosstalk              | *A. thaliana*                          | Laluk et al., 2012; Qiu et al., 2012     |
| *AtCAMTA5*     | Ethylene-induced senescence                                              | *A. thaliana*                          | Nie et al., 2012                         |
| *OsCBT*        | Negative regulation of resistance to *S. sclerotiorum*                  | *A. thaliana*                          | Rahman H. et al., 2016                   |
| *SlSR1, SlSR3L*| MEcPP mediated induction of the rapidly and transiently stress-responsive cis-element | *A. thaliana*                          | Benn et al., 2016                        |
| *SlSR1L*       | Glucosinolate metabolism and herbivory tolerance                        | *A. thaliana*                          | Laluk et al., 2012                      |
| *SISR1, SISR3L*| Freezing tolerance                                                       | *A. thaliana*                          | Kim et al., 2013                         |
| *VvCAMTA1; VvCAMTA3; VvCAMTA4; VvCAMTA5* | P receptive of stress and hormone signals                               | *Glycine max*                          | Wang et al., 2015                        |
| *MtCAMTAs*     | Nodule organogenesis                                                      | *Medicago truncatula*                  | Yang et al., 2015                        |
| *ZnCAMTA*      | Biotic/abiotic stress tolerance                                           | *Ze a mays*                            | Yue et al., 2015                         |
| *PtCAMTA*      | Biotic/abiotic stress management and ABA, SA, MeJA homeostasis           | *Populus trichocarpa*                  | Wei et al., 2017                         |
| *GhCAMTA2A.2; GhCAMTA7A* | Cotton fiber development                                                | *Gossypium hirsutum*                   | Pant et al., 2018                        |
| *FaCAMTA*      | Heat, cold, and salt stress                                              | *Fragaria ananassa*                    | Leng et al., 2015                        |
| *BnCAMTA*      | Stress-inducible and phytohormonal regulation                            | *Brassica napus*                       | Rahman H. et al., 2016                   |

(Continued)
In silico analysis revealed that PvuCAMTAs were involved in salt stress signaling and were further substantiated through RNA-seq and qRT-PCR analyses (Buyuk et al., 2019). In concurrence to the above study, PvuCAMTA1 has also been characterized to impart drought tolerance (Saedi et al., 2019). CAMTA gene family had also been well reported in citrus (Zhang et al., 2019). Nine CAMTA genes were found in the citrus (C. sinensis and Citrus clementina) genomes. Most of the CitCAMTAs were found to be highly conserved during evolution. All CitCAMTA genes (except CitCAMTA4) were expressed in minimum one plant tissue corroborating their involvement in leaf, root, stem, seed, cotyledon, and fruit development. Hormone and stress experiments revealed varying expression profiles of CitCAMTAs, indicating stress adaptation. Upon salt stress (0- to 24-h treatment), CitCAMTA1, CitCAMTA5, and CitCAMTA9 were significantly expressed. The expression of CitCAMTA7 first increased and then decreased as the time of salt treatment progressed. The expression of CitCAMTA3 was suppressed at all time points. For the remaining CitCAMTAs, i.e., CitCAMTA2, CitCAMTA6, and CitCAMTA8, there were no obvious changes observed. Upon dehydration stress (0- to 12-h treatment), CitCAMTA5, CitCAMTA6, and CitCAMTA8 levels slightly declined, whereas for CitCAMTA3 and CitCAMTA9, the expression levels were up-regulated reaching a maximum at 12 h of treatment. CitCAMTA1 and CitCAMTA2 showed moderate level of expression under dehydration stress. While most of the CitCAMTAs were responsive to salt and dehydration stress, CitCAMTA4 remain unaltered. Lastly, the authors investigated the involvement of CitCAMTAs in hormonal signaling (SA, ETH, IAA, 6-BA, ABA, MeJA, and GA3). All the CitCAMTAs (except CitCAMTA4) were responsive to at least one phytohormone. CitCAMTA1, CitCAMTA5, CitCAMTA7, and CitCAMTA9 positively correlated to all the hormones, whereas CitCAMTA3 showed a negative correlation. These observations led the authors to hypothesize the involvement of CitCAMTAs in abiotic stress pathways and hormonal regulation (Zhang et al., 2019). Genome-wide identification also led to the identification of 15 wheat CAMTA genes, which were classified into three groups A, B, and C containing seven, six, and two TaCAMTAs, respectively (Yang et al., 2020). Most of the TaCAMTAs contained stress-responsive cis-regulatory elements. All the 15 TaCAMTAs expressed differentially in various tissues and under different abiotic stresses. The expression analysis of TaCAMTA1-A and 1-D revealed that they were significantly expressed under drought, cold, heat, and salt stress. TaCAMTA genes belonging to the same homologous group had similar expression profiles under particular stress condition. For example, TaCAMTA1-A/B/D had almost similar expression profile under drought stress, TaCAMTA5-A/D under salt stress, and TaCAMTA1-A/B/D under heat stress. This observation implies that homologous TaCAMTA genes from the same group might share same functionality. However, few homologous TaCAMTA genes belonging to the same group displayed varying expression profiles under drought, cold, heat, and salinity stress. For instance, TaCAMTA3-A/D was significantly expressed upon cold stress, but the expression profiles of TaCAMTA1-B and TaCAMTA3-B were comparatively stable. This might be due to the functional differentiation in some homologous TaCAMTA genes. Additionally, in the study, it was depicted that 584 genes in the wheat genome can be regulated by using the CaM-encoding gene (TaCaM4-I) as a bait, for subsequent screening of cDNA library from P. tritici-infecte Triticum leaves. The EMSA results revealed that TaCaM4-I binds to TaCAMTA4 by the C-terminal CaMBD in Ca²⁺-dependent manner. Bimolecular fluorescence complementation (BiFC) analysis showed cytoplasm and nucleus as the probable interaction site of TaCAMTA4 and TaCAMA-1 (Wang et al., 2019). In a similar vein, nine CAMTA genes were identified in Flax (L. usitatissimum) upon genome-wide identification study. These nine CAMTA proteins were classified into three groups, depending on the phylogenetic analysis. Various hormonal (ABA and SA) and stress-related (drought, low temperature, and light) cis-regulatory elements were found to be enriched in promoter region of LuCAMTAs genes. In addition, the miRNA target analysis showed different miRNA families (miRN30, miRN9, miR2275, miR164, miR159, miR164, miRN15,
miR395, miR156, miR28, and miR164) as probable targets of the LuCAMTA genes (except LuCAMTA9) (Ali et al., 2020).

CONCLUSION AND FUTURE PERSPECTIVE

Improving crop yield and productivity for the ever-growing population has been a major challenge all over the world. The demand for ever-increasing food supply has put forth a tremendous pressure on the agriculture sector. The advent of new biotechnological tools and techniques has robustly contributed to crop improvement and horticultural science (Limera et al., 2017; Adlak et al., 2019). In this regard, several TFs, including CAMTA, had been identified and functionally characterized. Ever since the discovery of CAMTAs, its role had been comprehensively reported in developmental and stress biology in various model and crop plants (Table 1). The objective ahead is to understand the fundamental functionality and molecular mechanism behind CAMTA protein expression and downstream processing. A deeper understanding of the mechanisms underlying the involvement of Ca\(^{2+}\) and CaM in CAMTA transcript expression would provide great insights into its probable function in various tissues and response to environmental cues. In addition, deciphering the probable functions and mechanisms underlying the complex regulatory property of CAMTA proteins through its ability to transduce Ca\(^{2+}\) signatures via CaM can improve our knowledge of phenotypic plasticity in plants. However, because of the possible functional redundancy or at least overlapping functions, molecular characterization of individual CAMTA genes still poses a great challenge ahead for researchers. Rigorously developed CRISPR/Cas9-mediated genome editing techniques that rapidly generate mutations at multiple loci using one single-guide RNA will effectively help characterizing each of the gene family members. Nonetheless, the involvement of CAMTA as stress-related gene and its subsequent study in this direction would widen and provide vast knowledge for improvement in crop yield, productivity, and strategies to cope with adverse conditions in arid or semiarid regions of the world.

AUTHOR CONTRIBUTIONS

ZI drafted the manuscript. MSI helped in drawing the figures. SPS edited the manuscript. TB reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded using research grants from the Second Century Fund (C2F Post-Doc fellowship). The fellowship for ZI was supported by C2F, Chulalongkorn University.

ACKNOWLEDGMENTS

ZI is thankful to TB for providing the facilities and infrastructure.
Kim, M. C., Chung, W. S., Yun, D.-J., and Cho, M. J. (2009). Calcium and calmodulin-mediated regulation of gene expression in plants. Mol. Plant 2, 13–21. doi: 10.1093/mp/ssn091

Kim, Y., Gilmour, S. J., Chao, L., Park, S., and Thomashow, M. F. (2020). Arabidopsis CAMTA transcription factors regulate pipecolic acid biosynthesis and prcharging of immunity genes. Mol. Plant 13, 157–168. doi: 10.1016/j.molp.2019.11.001

Kim, Y., Park, S., Gilmour, S. J., and Thomashow, M. F. (2013). Roles of CAMTA transcription factors and calicic acid in confiuring the low-temperature transcriptome and freezing tolerance of Arabidopsis. Plant J. 75, 364–376. doi: 10.1111/pj.12005

Kim, Y. S., An, C., Park, S., Gilmour, S. J., Wang, L., Renna, L., et al. (2017). CAMTA-mediated regulation of calicic acid immunity pathway genes in Arabidopsis exposed to low temperature and pathogen infection. Plant Cell 29, 2465–2477. doi: 10.1105/tpc.16.00865

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. Mol. Cells 27, 563–570. doi: 10.1007/s10059-009-0081-4

Kordyum, E. (2003). Calcium signaling in plant cells in altered gravity. Adv. Space Res. 32, 1621–1630. doi: 10.1016/S0273-1177(03)00403-0

Kudla, J., Becker, D., Grill, E., Hedrich, R., Hippler, M., Kummer, U., et al. (2010). Characterization of a calmodulin-binding transcription activator (CAMTAs) to produce specific gene expression responses. New Phytol. 208, 174–187.

Lolle, S., Greef, C., Petersen, K., Roux, M., Jensen, M. K., Bresendorff, S., et al. (2017). Matching NLR immune receptors to autoimmunity in camtA3 mutants using antimorphic NLR alleles. Cell Host Microbe 21, 518–529.e4.

Luan, S. (2009). The CBL–CIPK network in plant calcium signaling. Trends Plant Sci. 14, 37–42. doi: 10.1016/t.psl.2008.10.005

Ma, W., and Berkowitz, G. A. (2007). The grateful dead: calcium and cell death in plant innate immunity. Cell Microbiol. 9, 2571–2585. doi: 10.1111/j.1462-5822.2007.01031.x

Manishankar, P., Wang, N., Köster, P., Alatar, A. A., and Kudla, J. (2018). Calcium signaling during salt stress and in the regulation of ion homeostasis. J. Exp. Bot. 69, 4215–4226. doi: 10.1093/jxb/ery201

Marques, D. N., Dos Reis, S. P., and De Souza, C. R. (2017). Plant NAC transcription factors responsive to abiotic stresses. Plant Gene 53, 2008–2015. doi: 10.1093/pcp/pcs143

Matsui, T., Efthimiadou, A., Bacopoulou, F., Vlachakis, D., Chrousos, G. P., and Eliopoulos, E. (2020). Transcription factors and evolution: an integral part of gene expression. World Acad. Sci. J. Life Sci. 12, 3–8.

Matsuda, N., Isono, T., and Sato, M. H. (2003). Arabidopsis CAMTA family proteins enhance V-PPase expression in pollen. Plant Cell Physiol. 44, 975–981. doi: 10.1093/pcp/pcg137

McCormack, E., Tsai, Y.-C., and Braam, J. (2005). Handling calcium signaling: Arabidopsis CaMs and CMLs. Trends Plant Sci. 10, 383–389. doi: 10.1016/t.psl.2005.07.001

Medvedev, S. (2005). Calcium signaling system in plants. Russ. J. Plant Physiol. 52, 249–270. doi: 10.1007/s11183-005-0038-1

Mitsis, T., Efthimiadou, A., Baboupolou, F., Glavachikis, D., Chrousos, G. P., and Eliopoulos, E. (2020). Calcium imbalance: a universal regulator of plant growth and development. Plant Physiol. 175, 702–708. doi: 10.1104/pp.120.042069

Mi, S., and Mn, W. (2008). Ca2+ and calmodulin-dependent protein kinases. Cell. Mol. Life Sci. 65, 2637–2657.

Müller, C. W., Rey, F. A., Sodeoka, M., Verdine, G. L., and Harrison, S. C. (1995). Structure of the NP- FAP homodimer bound to DNA. Nature 373, 311–317.

Nakamura, K., Tran, L. S. P., Van Nguyen, D., Fujita, M., Maruyama, K., Todaka, D., et al. (2007). Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. Plant J. 51, 617–630. doi: 10.1111/j.1365-313x.2007.03168.x

Nakata, M., Yusa, T., Takahashi, Y., and Ishida, S. (2009). CDPK1, a calcium-dependent protein kinase, regulates transcriptional activator RSG in response to gibberellins. Plant Signal. Behav. 4, 372–374. doi: 10.4161/pbs.4.5.8229

Nakayama, S., Kasahashi, H., and Kretzinger, R. (2000). “Evolution of EF-hand proteins,” in Calcium Homeostasis, eds E. Carafoli, and J. Krebs (Berlin: Springer), 29–58. doi: 10.1007/978-3-642-58306-3_2
Nie, H., Zhao, C., Wu, G., Wu, Y., Chen, Y., and Tang, D. (2012). SRI, a calmodulin-binding transcription factor, modulates plant defense and ethylene-induced senescence by directly regulating NDR1 and EIN3. *Plant Physiol.* 158, 1847–1859. doi: 10.1104/pp.111.192310

Noman, M., Jameel, A., Qiang, W.-D., Ahmad, N., Liu, W.-C., Wang, F.-W., et al. (2019). Overexpression of GmCAMTA12 enhanced drought tolerance in arabidopsis and soybean. *Int. J. Mol. Sci.* 20:4849. doi: 10.3390/ijms20194849

Pandey, N., Ranjan, A., Pant, P., Tripathi, R. K., Attek, F., Pandey, H. P., et al. (2013). CAMTA 1 regulates drought responses in *Arabidopsis thaliana*. *BMC Genomics* 14:216. doi: 10.1186/1471-2164-14-216

Pant, P., Iqbal, Z., Pandey, B. K., and Sawant, S. V. (2018). Genome-wide comparative and evolutionary analysis of Calmodulin-binding Transcription Activator (CAMTA) family in Gossypium species. *Sci. Rep.* 8:5573.

Park, C. Y., Lee, J. H., Yoo, J. H., Moon, B. C., Choi, M. S., Kang, Y. H., et al. (2005). SR1, a calmodulin-binding transcription factors interact with calmodulin. *FEBS Lett.* 579, 1545–1550. doi: 10.1016/j.febslet.2005.01.057

Phukan, U. J., Jeena, G. S., and Shukla, R. K. (2016). WRKY transcription factors: molecular regulation and stress responses in plants. *Front. Plant Sci.* 7:670. doi: 10.3389/fpls.2016.00760

Poovaiyah, B. W., Reddy, A. S. N., and Feldman, L. (1993). Calcium and signal transduction in plants. *Crit. Rev. Plant Sci.* 12, 185–211. doi: 10.1080/10603169309361780

Popescu, S. C., Popescu, G. V., Bachan, S., Zhang, Z., Seay, M., Gerstein, M., et al. (2007). Differential binding of calmodulin-related proteins to their targets revealed through high-density Arabidopsis protein microarrays. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4743–4753. doi: 10.1073/pnas.061615104

Puranik, S., Sahu, P. P., Srivastava, P. S., and Prasad, M. (2012). NAC proteins: regulation and role in stress tolerance. *Trends Plant Sci.* 17, 369–381. doi: 10.1016/j.tplants.2012.02.004

Qi, Y., Xi, J., Du, L., Suttle, J. C., and Poovaiyah, B. W. (2012). Coupling calcium/calmodulin-mediated signaling and herbivore-induced plant response through calmodulin-binding transcription factor ASR1/CAMTA3. *Plant Mol. Biol.* 79, 89–99. doi: 10.1007/s11103-012-9896-z

Roy, S. (2016). Function of MYB domain transcription factors in abiotic stress in plants. *Front. Plant Sci.* 7, 369–381. doi: 10.3389/fpls.2016.00609

Rubtsov, A. N., Reddy, R. V. S., and Golovkin, M. (2000). A calmodulin binding protein from Arabidopsis is induced by ethylene and contains a DNA-binding motif. *Biochem. Biophys. Res. Commun.* 279, 762–769. doi: 10.1006/bbrc.2000.4032

Scott, I. M., Clarke, S. M., Wood, J. E., and Mur, L. A. (2004). Salicylate accumulation inhibits growth at chilling temperature in Arabidopsis. *Plant Physiol.* 135, 1040–1049. doi: 10.1104/pp.104.041293

Shangguan, L., Wang, X., Leng, X., Liu, D., Ren, G., Tao, R., et al. (2014). Identification and bioinformatic analysis of signal responsive/calmodulin-binding transcription activator genes models in *Vitis vinifera*. *Mol. Biol. Rep.* 41, 2937–2949. doi: 10.1007/s11033-014-3150-5

Shi, H., Ye, T., Zhong, B., Liu, X., and Chan, Z. (2014). Comparative proteomic and metabolomic analyses reveal mechanisms of improved cold stress tolerance in bermudagrass (*Cynodon dactylon* (L.) Pers.) by exogenous calcium. *J. Integr. Plant Biol.* 56, 1064–1079. doi: 10.1111/jipb.12167

Song, K., Backs, J., McAnally, J., Xi, Q., Gerard, R. D., Richardson, J. A., et al. (2006). The transcriptional coactivator CAMTA2 stimulates cardiac growth by opposing class II histone deacetylases. *Cell* 125, 453–466. doi: 10.1016/j.cell.2006.02.048

Stracke, R., Werber, M., and Weishhaar, B. (2001). The R2R3-MYB gene family in *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* 4, 447–456. doi: 10.1016/s1369-5266(00)00199-0

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-hydroxypropeic acid by modulating the expression of SARD1 and CBP60g. *Mol. Plant* 13, 144–156. doi: 10.1016/j.molp.2019.10.016

Sun, X., Wang, Y., and Sui, N. (2018). Transcriptional regulation of bHLH during plant response to stress. *Biochem. Biophys. Res. Commun.* 503, 397–401. doi: 10.1016/j.bbrc.2018.07.123

Swarbreck, S. M., Colaco, R., and Davies, J. M. (2013). Plant calcium-permeable channels. *Plant Biol.* 163, 514–522. doi: 10.1104/pp.113.220855

Teige, M. (2019). Chloroplasts use calcium signals to call for help under heat stress. *Plant Cell Physiol.* 60, 492–493. doi: 10.1093/pcp/pct039

Thor, K. (2019). Calcium—nutrient and messenger. *Front. Plant Sci.* 10:440. doi: 10.3389/fpls.2019.00440

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

Tian, W., Wang, C., Gao, Q., Li, L., and Luan, S. (2020). Calcium spikes, waves and oscillations in plant development and biotic interactions. *Nat. Plants* 6, 1–10. doi: 10.1038/s41477-020-0415-4

Toikia, M., Kobayashi, Y., Saito, T., Kobayashi, M., Iuchi, S., Nomoto, M., et al. (2015). SARD1, a calmodulin binding transcription activator2, and other transcription factors are involved in aluminum-activated dwarfing in rice. *Front. Plant Sci.* 6, 233. doi: 10.3389/fpls.2015.00216

Tokizawa, M., Kobayashi, Y., Saito, T., Kobayashi, M., Iuchi, S., Nomoto, M., et al. (2019). Calcium signaling and salt tolerance are diversely entwined in bermudagrass (*Cynodon dactylon* (L.) Pers.) by exogenous calcium. *J. Integr. Plant Biol.* 56, 1064–1079. doi: 10.1111/jipb.12167

Totten, J. L., and Scott, I. M. (2002). Calcium/calmodulin-mediated signaling and herbivore-induced plant response through calmodulin-binding transcription factor ASR1/CAMTA3. *Plant Mol. Biol.* 5266(00)00199-0

Yi, M., Tang, J., Yuan, X., and Deng, W. (2016). Calcium−Calmodulin Triggers CAMTA Transcriptional Machinery. *Front. Plant Sci.* 7, 670. doi: 10.3389/fpls.2016.00760

Zhou, Z., Wei, Y., Wang, Y., and Luan, S. (2016). The transcription factor CAMTA2 is induced by ethylene and contains a DNA-binding motif. *Biochem. Biophys. Res. Commun.* 472, 151–157. doi: 10.1016/j.bbrc.2016.02.048

Zhong, Y., and Deng, W. (2018). Calcium−Calmodulin−CAMTA Transcriptional Machinery Contributes to PAMP Triggered Immunity and Resistance to *Sclerotinia sclerotiorum*. *Front. Plant Sci.* 7:670. doi: 10.3389/fpls.2016.00760
NAC transcription factors that bind to a drought-responsive cis-element in the early responsive to dehydration stress 1 promoter. Plant Cell 16, 2481–2498. doi:10.1105/tpc.104.022699

Tripathi, D. K., Singh, V. P., Chauhan, D. K., Sharma, S., Prasad, S. M., Dubey, N. K., et al. (2020). Plant Life Under Changing Environment: Responses and Management. Cambridge, MA: Academic Press.

Tupler, R., Perini, G., and Green, M. R. (2001). Expressing the human genome. Nature 409, 832–835. doi:10.1038/35075011

Tuteja, N., and Mahajan, S. (2007). Calcium signaling network in plants: an overview. Plant Signal. Behav. 2, 79–85. doi: 10.4161/psb.2.2.4176

Vanneste, S., and Friml, J. (2013). Calcium: the missing link in auxin action. Plants 2, 650–675. doi: 10.3390/plants2040650

Villalobo, A., González-Muñoz, M., and Berchtold, M. W. (2019). Proteins with calmodulin-like domains: structures and functional roles. Cell. Mol. Life Sci. 76, 2299–2328. doi: 10.1007/s00018-019-03062-z

Vogel, J. T., Zarka, D. G., Van Buskirk, H. A., Fowler, S. G., and Thomashow, M. F. (2005). Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis. Plant J. 41, 195–211. doi: 10.1111/j.1365-313x.2004.02288.x

Walley, J. W., and Dehesh, K. (2010). Molecular mechanisms regulating rapid stress signaling networks in Arabidopsis. J. Integr. Plant Biol. 52, 354–359. doi:10.1111/j.1744-7909.2010.00940.x

Wang, C., Shang, J.-X., Chen, Q.-X., Osei-Prieto, J. A., Bai, M.-Y., Yang, Y., et al. (2013). Identification of BZR1-interacting proteins as potential components of the brassinosteroid signaling pathway in Arabidopsis through tandem affinity purification. Mol. Cell. Proteomics 12, 3653–3665. doi: 10.1074/mcp.m13.029256

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 Soil 386, 205–221. doi: 10.1007/s11104-014-2267-6

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. Sci. Rep. 9:641.

Wang, Z., Jia, C., Wang, J.-Y., Mao, X.-X., Liu, J.-H., Chen, C., et al. (2020). Genome-wide analysis of basic helix-loop-helix transcription factors to elucidate candidate genes related to fruit ripening and stress in Banana (Musa acuminata L. AAA Group, cv. Cavendish). Front. Plant Sci. 11:650. doi: 10.3389/fpls.2020.00650

Wei, M., Xu, X., and Li, C. (2017). Identification and expression of CAMTA genes in Populus trichocarpa under biotic and abiotic stress. Sci. Rep. 7:17910.

Wu, L., Sadhukhan, A., Kobayashi, Y., Ogo, N., Tokizawa, M., Agrahari, R. K., et al. (2019). Involvement of phosphatidylinositol metabolism in aluminum-induced malate secretion in Arabidopsis. J. Exp. Bot. 70, 3329–3342. doi: 10.1093/jxb/erz179

Xu, J., Qiu, Y., Du, L., and Poovaiah, B. W. (2012). Plant-specific trihelix transcription factor AtGT2L interacts with calcium/calmodulin and responds to cold and salt stresses. Plant Sci. 185, 274–280. doi:10.1016/j.plantsci.2011.11.013

Xiong, T.-C., Bourque, S., Lecourieux, D., Amelot, G., Grat, S., Brière, C., et al. (2015). Genome-wide identification of calcium/calmodulin-regulated SR/CAMTA genes and their expression analysis under stress and hormone treatments. J. Hortic. Sci. Biotechnol. 94, 331–340. doi: 10.1080/14630316.2018.1504631

Zhang, T., Zhao, Y., Wang, Y., Liu, Z., and Gao, C. (2018). Comprehensive analysis of MYB gene family and their expressions under abiotic stresses and hormone treatments in Tamarix hispida. Front. Plant Sci. 9:1303. doi: 10.3389/fpls.2018.01303

Zhu, L., Jin, X., Xie, Q., Yao, Q., Wang, X., and Li, H. (2018). Calcium-dependent protein kinase family genes involved in ethylene-induced natural rubber production in different Hevea brasiilensis cultivars. Int. J. Mol. Sci. 19:947. doi: 10.3390/ijms1909947

Zhu, Y., Yan, J., Liu, W., Liu, L., Sheng, Y., Sun, Y., et al. (2016). Phosphorylation of a NAC transcription factor by ZmCaMK regulates abscisic acid-induced antioxidant defense in maize. Plant Physiol. 171, 1651–1664. doi: 10.1104/pp.16.00168

Zielinski, R. E. (1998). Calmodulin and calmodulin-binding proteins in plants. Annu. Rev. Plant Biol. 49, 697–725. doi: 10.1146/annurev.arplant.49.1.697

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Iqbal, Shariq Iqbal, Singh and Buaboocha. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.