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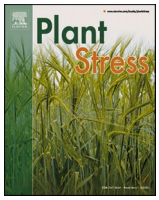

Genome-wide analysis of the calmodulin-binding transcription activator (*CAMTA*) gene family in *Sesamum indicum* L., and its role in abiotic stress tolerance traits

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ABSTRACT

Calmodulin-binding transcription activator (CAMTA) is one of the key transcription factor families possessing calcium receptors (calmodulins, CaM). It modulates the expression levels of genes associated with ontogeny and various biotic and abiotic stress factors. The *CAMTA* family genes were known to be involved in different abiotic stress in several crop species. However, their functional relevance in sesame remains unexplored. To understand the role of *CAMTA* in stress tolerance in sesame, we performed a genome-wide analysis to identify the members of the *SiCAMTA* gene family. We have identified and reported here the five *SiCAMTA* genes localized on four chromosomes within the sesame genome. *In silico* analysis of the putative 2-kilobase (kb) promoter regions for these five *SiCAMTA* genes showed that phytohormone and stress response-related cis-elements were predominated in *SiCAMTA2* and *SiCAMTA5*. Also, we studied its modulated expression levels, with special reference to drought and waterlogging stress. It revealed that the *SiCAMTA5* and *SiCAMTA2* genes were the most responsive to the studied stress factors. The target prediction and network analysis suggested that *SiCAMTAs* could bind the CGCG *cis* element in the target gene promoters and predicted 1202 *SiCAMTA* target genes in the sesame genome, including abiotic stress-responsive genes *viz. LEA, PIP1–2, PPO1, SAP, ARF17*, and *GA3OX1*. These findings were validated using qPCR analysis for five *CAMTA* and 10 *CAMTA* target genes and establish a foundation for future functional research of *SiCAMTA* genes towards sesame stress tolerance.

1. Introduction

Calcium (Ca^{2+}) ions act as a pivotal secondary messenger in the signal transmission process, thereby mediating plants' responses to environmental and developmental cues (Zhang et al., 2014; Furio et al., 2020). Fluctuations in intra-cellular Ca^{2+} levels are sensed and interpreted by several Ca^{2+}-binding proteins that act as signal sensors (Kudla et al., 2018; Iqbal et al., 2020). In plants, calmodulin/calmodulin-like proteins (CaMs/CMLs) are the well characterized Ca^{2+} sensors that perform their biological functions *via* multiple interactions with calmodulin-binding proteins (CaMBPs) such as protein kinases, ion channels, enzymes, and transcription factors (TFs) (Rahman et al., 2016; Wei et al., 2017). It is reported that more than 90 TFs, including calmodulin-binding transcription activators (CAMTAs), were regulated through CaMs and CMLs (Yang et al., 2015; Kakar et al., 2018). CG-1 domain, TIG domain, Ankyrin repeat (ANK), CaMBD, and IQ motifs (IQXXXRGXXXR) were the key conserved functional domains of the CAMTA proteins (Finkler et al., 2007). These domains are involved in various specific functions. CG-1 domain implicated DNA binding at the N-terminal, TIG domain engaged in non-specific DNA binding, while ANK is responsible for mediating protein-protein interactions, CaMBD (CaM-binding domain) is involved in the interaction of CaM with CAMTA, and IQ motifs interact with CaM and CaM-like proteins (Yang et al., 2012; Rahman et al., 2016; Büyük et al., 2019). The *CAMTA* was first identified as an ethylene-responsive gene in tobacco (Yang and Poovaiah, 2000). Since then, the *CAMTA* gene family has been identified and reported in numerous plant species, such as *Arabidopsis thaliana* (6), rice (7), rape (18), soybean (15), maize (9), poplar (7), citrus (9), wheat

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(15), and other plants (Bouché et al., 2002; Choi et al., 2005; Rahman et al., 2016; Wang et al., 2015; Yue et al., 2015; Wei et al., 2017; Zhang et al., 2019; Yang et al., 2020). Among them, several *CAMTA* family genes have been shown to mediate transcriptional regulation and are crucial for crosstalk between multiple signal transduction pathways in response to biotic and abiotic stresses (Yue et al., 2015; Chung et al., 2020; Noman et al., 2019). Its expression level varies among plant species in response to different stresses (Wei et al., 2017). Also, their regulatory mechanisms in abiotic stress tolerance, such as drought and cold, have been reasonably understood in plants (Doherty et al., 2009; Kim et al., 2013; Pandey et al., 2013). In *A. thaliana*, the expression of all six *AtCAMTAs* was rapidly influenced by various abiotic stress factors. For instance, *AtCAMTA1* and *AtCAMTA2* interact with *AtCAMTA3* to improve freezing tolerance by inducing the expression of *CBF* genes (Doherty et al., 2009; Kim et al., 2013; Novikova et al., 2020); while *AtCAMTA1* promotes drought tolerance (Pandey et al., 2013; Meer et al., 2019; Saeidi et al., 2019). The *BnCAMTA3* gene of *Brassica napus* (oilseed rape) has been found to play an important role in cold tolerance and disease resistance (Rahman et al., 2016). A recent study in soybean showed that *GmCAMTA2* and *GmCAMTA8* synchronize circadian regulation during their developmental processes and drought stress (Baek et al., 2023). Also, the higher expression of the *CAMTA4* gene has been linked to the development of lysigenous aerenchyma tissues in the primary roots of the oil palm (*Elaeis guineensis* Jacq.), which facilitates the flow of oxygen for survival under waterlogged conditions (Nuanlaong et al., 2021). The potential target genes of CAMTAs can be predicted through the analysis of the presence of *CAMTA* specific cis-acting elements in the promoter regions (Yue et al., 2015; Noman et al., 2019; Sun et al., 2020). In the wheat genome, about 584 genes were predicted as potential target genes of *TaCAMTAs* and were found to regulate drought stress response during the seedling stage of wheat (D. Wang et al., 2022).

Sesame (*Sesamum indicum* L.) is a globally important oilseed crop grown primarily in tropical and subtropical regions that provides high-quality nutrients and nutraceuticals beneficial to human health (Myint et al., 2020; Wei et al., 2022). Abiotic stresses such as drought, waterlogging, salt, and heat have an impact on sesame productivity and seed quality (Kermani et al., 2019; Dossa et al., 2019). Among them, drought and waterlogging were the most adverse environmental factors that impair sesame by affecting various biochemical and physiological processes (Wang et al., 2016; Dossa et al., 2017; Anee et al., 2019). In recent years, with the availability of genome-scale information, the gene function and role of TFs have been documented in sesame with special reference to abiotic stresses (Dossa et al., 2017, 2019; Wang et al., 2021; Mmadi et al., 2017; Li et al., 2017). However, despite the acknowledged importance of CAMTA TF in response to various abiotic stress in several crop species, the systematic genome-wide investigation and role of *CAMTA* gene family in response to abiotic stresses have not been explored in sesame.

The present study aimed to augment our understanding of the structure and function of the *CAMTA* gene family in association with abiotic stress factors in sesame. Here, we have identified five *SiCAMTA* genes in the *S. indicum cv. Baizhima* genome (D. Wang et al., 2022); their physico-chemical properties, gene structure and distribution, and cis-elements were studied. The phylogenetic and synteny analyses were also performed to gain insight into their evolutionary relationships. Using available RNA-seq. data, we quantified and analyzed the expression profiles of five *SiCAMTA* genes and their target genes in response to drought and waterlogging stress. Our findings reveal the role, importance, and molecular mechanism of *SiCAMTA* gene family members in imparting drought and waterlogging stress tolerance through modulating various target genes.

2. Materials and methods

2.1. Identification of CAMTA genes in Sesamum indicum

The protein sequences of six *Arabidopsis thaliana* CAMTA proteins were retrieved from the phytozome database (https://phytozome-next.jgi.doe.gov/) and used as queries to perform Blastp (E-value: 1e-6) (Camacho et al., 2009) searches within the genome of *S. indicum cv. Baizhima* acquired from the Fig Share Database (https://doi.org/10.6084/m9.figshare.21151948) (D. Wang et al., 2022). The Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/cdd/) (Marchler-Bauer et al., 2010), Simple Modular Architecture Research Tool (SMART) (http://smart.emblheidelberg.de/) (Letunic and Bork, 2018), and Pfam (http://pfam.xfam.org/) (El-Gebali et al., 2019) were used to further verify the presence of conserved CAMTA functional domains such as CG-1 domain (PF03859), IPT/TIG (PF01833), Ank repeats (PF00023), and IQ motifs (PF00612). Redundant sesame CAMTA (SiCAMTA) proteins were removed, and the remaining putative SiCAMTA protein sequences containing the CG-1 domain, TIG domain, Ank domain, and IQ domain were recognized as *SiCAMTA* gene family members and retained for subsequent analysis. The CaMBD domain (calmodulin-binding domain) of SiCAMTA proteins was identified manually using the conserved motif sequence (WXVX(2)LXKX(2)[LF]RWRX[KR]X(3)[FL]RX) of the *A. thaliana* CAMTA protein (Pant et al., 2018). Then the domains were further visualized using illustrator for the biological sequences (IBS) web server (http://ibs.biocuckoo.org/online.php) (Liu et al., 2015). The CaMBD sequence logo of the SiCAMTAs was visualized by TBtools-II (Chen et al., 2023).

2.2. Physiochemical properties prediction

Sesame CAMTA proteins' physicochemical properties, such as theoretical isoelectric point (pI), molecular weight (Mw), grand average of hydropathicity, (GRAVY) instability index, and aliphatic index of sesame, were predicted using the ProtParam tool on the ExPASy server (https://web.expasy.org/protparam/) (Gasteiger et al., 2005). The subcellular location and transmembrane domains in SiCAMTA proteins were predicted using DeepLoc-2.0 (https://services.healthtech.dtu.dk/service.php?DeepLoc-2.0) (Thumuluri et al., 2022) and the DeepTMHMM (v1.0.24) (https://dtu.biolib.com/DeepTMHMM) (Hallgren et al., 2022).

2.3. Phylogenetic relationship analysis

The protein sequences of CAMTA family members from different crops, including *Oryza sativa, A. thaliana*, and eight oilseed crops, *Glycine* max, *Arachis hypogea, Brassica rapa, Gossypium hirsutum, Helianthus annuus, Linum usitatissimum, Sesamum indicum,* and *Ricinus communis* (**Supplementary Table S1**), were aligned using the ClustalW (v2.1) program (Thompson et al., 1994). The dendrogram was constructed using MEGA software (MEGA-X) with the neighbor-joining (NJ) method and 1000 bootstrap replications (Kumar et al., 2018). Further, the dendrogram was visualized using the iTOL (v5) web server (https://itol.embl.de/) (Letunic and Bork, 2021).

2.4. Conserved motif and exon-intron analysis

The conserved motifs were predicted by the MEME (Multiple Expectation Maximization for Motif Elicitation) tool (http://meme-suite.org/tools/meme) (Bailey et al., 2006). Using the MEME suite (v5.4.1), the motifs were searched using default parameters with 'number of motifs' to 15. The distribution of conserved motifs of SiCAMTAs was visualized using TBtools-II (Chen et al., 2023). The genomic and coding sequences (CDSs) of five *SiCAMTA* genes were submitted into the Gene Structure Display Server 2.0 (GSDS v2.0, http://gsds.gao-lab.org/) (Hu et al., 2015) to predict their exon-intron

structures.

2.5. Chromosomal localization, Ka/Ks ratios, gene duplication and synteny analysis

The chromosomal positions for five *SiCAMTA* genes were obtained from the *S. indicum cv. Baizhima* genome. The positional localization of the *SiCAMTA* gene and collinearity analysis within *S. indicum cv. Baizhima* genome was performed and visualized using TBtools-II software. The synonymous substitution rate (Ks) values, nonsynonymous substitution rate (Ka) values, and the Ka/Ks ratio were used to assess selection history and duplication events (Nekrutenko et al., 2002). The number of synonymous (Ks) and nonsynonymous (Ka) substitutions of duplicated *SiCAMTA* genes was computed using TBtools-II software. The formula for calculating the time (T) of duplication events in sesame was $T = Ks/(2 \times 6.5 \times 10^{-9})*10^{-6}$ million years ago (Mya) (Wang et al., 2014; Dossa et al., 2016). For synteny analysis, the genome fasta (genome.fa) and genome feature file (GFF3) of *S. indicum cv. Baizhima* (https://doi.org/10.6084/m9.figshare.21151948), *O. sativa (Osativa_v7.0), A. thaliana*, and *B. rapa* (*BrapaFPsc_v1.3*) (https://phytozome-next.jgi.doe.gov/) were obtained from the respective databases. Homologous gene pairs were identified using the MCScanX program of TBtools-II. The comparative synteny map of *S. indicum* associated with *A. thaliana, O. sativa*, and *B. rapa* genomes was accomplished and visualized by using the multiple synteny plot tool of TBtools-II (Chen et al., 2023).

2.6. Cis-regulatory element analysis for putative promoters of SiCAMTA genes

The putative promoter sequences, 2000 bp sequences upstream of the transcription start site of *SiCAMTA* genes, were retrieved from *S. indicum cv. Baizhima* using TBtools-II (Chen et al., 2023). The sequences were submitted to the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) to predict and analyze cis-regulatory elements related to plant growth and development, hormones, stress, and light responses in the promotor regions of *SiCAMTA* genes (Lescot et al., 2002).

2.7. Expression profiling of SiCAMTA genes under drought, and waterlogging

The expression level of the identified five *SiCAMTA* genes in response to drought and waterlogging stress was obtained using previously developed transcriptome data (Dossa et al., 2019; Wang et al., 2021). Briefly, the transcriptome data was downloaded for four drought treatments that include control (d0), soil moisture 15 % vwc (d1), soil moisture 9 % vwc (d2), and soil moisture 6 % vwc (d3) for two sesame accessions (ZZM0635 drought-tolerant; and ZZM4782 drought-sensitive) (Dossa et al., 2019). Similarly, transcriptome data of two sesame genotypes, ZZM2541 (R2G, waterlogging tolerant) and Ezhi No. 2 (EG, waterlogging sensitive), were downloaded for four waterlogging stress treatment conditions, such as at 0 h, 12 h, 24 h, and 48 h (Wang et al., 2021). Further, a reference-based transcriptome analysis was performed using a recently published chromosome-level genome assembly of *S. indicum cv. Baizhima* (D. Wang et al., 2022) using the 'Tuxedo' RNA-seq. pipeline as described in our previous study (Nawade et al., 2022). The transcripts exhibiting differences of at least twofold with an FDR value ≤ 0.05 were identified as significantly differentially expressed genes. The heatmap was constructed with log2-based expression fold-changes using TBtools-II software (Chen et al., 2023).

2.8. SiCAMTA's target gene identification

The prediction of the target genes of *SiCAMTA* genes was performed as described earlier (Wang et al., 2021). The CAMTA family transcription factor specifically binds to the CGCG box in the promoter region of target genes (Yang and Poovaiah, 2002). To find the cis-regulatory CAMTA binding motifs in the promoters of the genes regulated by CAMTA, the binding motifs of CAMTA (AAAG**CGCG**TGAA, C**CGCG**T, and AC**CGCG**T) were first downloaded from the JASPAR website (http://jaspar.genereg.net/) (Khan et al., 2018). Then, 2000 bp upstream sequences from the transcription start site of the whole genome set were retrieved from the sesame (*S. indicum cv. Baizhima*) genome using TBtools-II (M. Wang et al., 2022). These promoter regions were scanned for the presence of the CAMTA binding motif (CGCG box) using the FIMO tool (v5.0.3) (Grant et al., 2011) with a threshold ($p < 10^{-6}$). The genes with the CGCG box were considered potential target genes that were being regulated by CAMTA.

2.9. Protein-protein interaction network

To construct a protein interaction network, SiCAMTA protein orthologs in *A. thaliana* were obtained using the online STRING database (v12.0; http://string-db.org) (Szklarczyk et al., 2010). The protein interaction map was visualized and analyzed using Cytoscape (v3.10.1) (Shannon et al., 2003) with a confidence (score) cutoff value of 0.40. Each node within the interaction map represents proteins, and the thickness of the edges indicates the intensity of physical interaction between two nodes. In this interactome network, direct interactions of SiCAMTA with other proteins are highlighted in yellow color, whereas the edges are colored according to the representative color of the source node. While nodes in cyan with grey-colored edges represented indirect interactions.

2.10. Plant material, drought, and waterlogging stress treatments

The sesame accessions, IC129772 and EC350648 (drought-tolerant), GT-10 and Thilak (drought-sensitive) (Vishwakarma et al., 2024), EC334977 and EC334970 (waterlogging tolerant) and IC129289, and IC131542 (waterlogging sensitive) (Shah et al., 2024) were used for drought and waterlogging stress treatment for gene expression analysis. A total of 15 seeds (3 × 5) of each accessions were sown in a seedling tray containing soilrite mixture and provided with half-strength Hoagland solution and allowed to grow for 14-days in ambient condition. Further, drought and waterlogging treatments were performed using 14-day old seedlings. The drought stress was induced using 15 % (w/v) PEG6000/water solution for 48 hrs. In control condition, 10 mL distilled water while in stress condition 10 mL of 15 % PEG was added to each box of seedling tray. For waterlogging stress 14-day old seedling tray was placed in an open container filled with water sufficient to dip seedling at least 3–5 cm from soil surface for a duration of 48 h of waterlogging condition. The samples were collected at 0 h and 48 h after drought and waterlogging treatment for genes' differential expression analysis using a qRT-PCR (Bio-RAD CFX96 Real-Time System, USA). Hence collected samples were snap frozen in liquid nitrogen and stored at −80 °C until use. For qRT-PCR analysis, three technical replicates of pooled single biological replicate from two individual plants were used. In gene expression studies, to maximize the sample size and statistical power, pooling of samples from multiple independent plants are commonly used (Rego et al., 2019; Moebes et al., 2022).

2.11. qRT-PCR based expression analysis of SiCAMTA and its target genes under drought and waterlogging stress

The total RNA was extracted from 100 mg tissue samples that were collected and preserved in −80 °C from our experiments, at 0 hrs (control) and 48 hrs drought and waterlogging stress treated seedling using 1 ml of GeneZol CT RNA Extraction Reagent (Catalog no. PG-100,103; Puregene, Genetix Biotech Asia) according to manufacturer's protocol. cDNA was synthesized from 1 µg of total RNA using PrimeScript^{tm} first strand cDNA synthesis Kit (TaKaRa) with oligo dT primer. A total of 5 *SiCAMTA* and 10 target genes were selected for qRT-based

Table 1
Summary of *CAMTA* genes identified in sesame genome, and their physicochemical properties.

Gene ID	Gene name	Chromosome location	*A. thaliana* homologs	Protein								
				Length (aa[a])	pI[b]	MW[c] (kDa)	GRAVY[d]	AI[e]	II[f]	Stable/ unstable	NLS[g]	TMDs[h]	Loc[i]
Sesame18023	*SiCAMTA2*	chr10	*AtCAMTA2*	1021	5.60	114.19	−0.486	80.83	46.45	Unstable	Yes	NO	Nucleus
Sesame23827	*SiCAMTA3*	chr13	*AtCAMTA3*	1111	5.73	124.41	−0.584	77.35	44.49	Unstable	Yes	NO	Nucleus
Sesame13828	*SiCAMTA4a*	chr8	*AtCAMTA4*	940	6.17	105.37	−0.53	78.24	52.52	Unstable	Yes	NO	Nucleus
Sesame03716	*SiCAMTA4b*	chr2	*AtCAMTA4*	962	6.27	107.99	−0.485	82.22	53.37	Unstable	Yes	NO	Nucleus
Sesame19210	*SiCAMTA5*	chr10	*AtCAMTA5*	929	6.78	105.17	−0.516	76.95	44.37	Unstable	Yes	NO	Nucleus

[a] Amino acid
[b] Theoretical pI
[c] Molecular weight.
[d] Grand average of hydropathicity (GRAVY).
[e] Aliphatic index.
[f] Instability index.
[g] Nuclear localization signal.
[h] transmembrane domains.
[i] subcellularlocalization
Note: *SiCAMTA* genes were named according to their homologs in *A. thaliana*.

expression analysis during drought and waterlogging stress for validation. Details of gene and their specific primer for qRT-PCR designed using IDT software (https://www.idtdna.com/pages/tools/primerquest) were provided in **Supplementary Table S2**. All primer pairs were initially standardized through a semi-quantitative PCR for the presence of a single amplicon of the expected size for each gene and were electrophoretically verified using appropriate markers. The qRT-PCR was performed using 5X HOT FIREPol Evagreen qPCR supermix (Solis Biodyne) on Real-Time PCR (Bio-RAD CFX96 Real-Time System, USA). The PCR reaction was set in 10 µl consisting of 2 µl of 5X HOT FIREPol Evagreen qPCR supermix, 1.5 µl (~100 ng) of template cDNA, 2.5 µM each forward and reverse primers and made up to final volume using nuclease free water. The cycling parameters for qRT-PCR were as following: Initial activation 95 °C 12 min, 40 cycles of denaturation 95 °C for 15 s, annealing 60 °C for 20 s, and elongation 72 °C 20 s; and ended with a melt curve (65.0 to 95 °C at 0.5 °C/0.05 s increments) analysis. For normalization, sesame ubiquitin gene (*SiUBQ*) was used as a reference gene. The normalized relative expression levels of *SiCAMTA* and their target genes between control and treated conditions were calculated using $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008). Statistical analysis was conducted using one-way ANOVA and followed by Duncan's range test in SPSS 14.0. Different lowercase letters denote significant differences in gene expression level ($p \leq 0.05$).

3. Results

3.1. Genome-wide identification of CAMTA genes in S. indicum

A total of five putative *CAMTA* genes were identified in sesame (*SiCAMTAs*) using the Blastp program. The identified *SiCAMTA* genes were named *SiCAMTA2, SiCAMTA3, SiCAMTA4a, SiCAMTA4b*, and *SiCAMTA5* based on their corresponding *A. thaliana* homologs (Bouche et al., 2002). Details of five *SiCAMTA* genes, such as gene names, gene IDs, chromosome locations, protein length, molecular weight (MW), isoelectric point (pI), aliphatic index (AI), instability index (II), grand average of hydropathicity (GRAVY values), signal peptide, transmembrane domains, and subcellular localization of SiCAMTAs, are summarized in Table 1. The isoelectric point (pI) ranged from 5.60 (SiCAMTA2) to 6.78 (SiCAMTA5), while the predicted molecular mass varied from 105.17 kDa (SiCAMTA5) to 124.41 kDa (SiCAMTA3). The predicted length (amino acid) of SiCAMTA proteins varied from 929 (SiCAMTA5) to 1111 (SiCAMTA3), resulting in molecular weight variations (Table 1). The predicted aliphatic index suggested the high thermal stability of SiCAMTAs (Table 1). The negative GRAVY values and instability index value above 40 indicate that all SiCAMTAs are hydrophilic and unstable (Table 1). Besides, all five SiCAMTAs were predicted to contain no TMDs. Moreover, all five SiCAMTAs were found to have a nuclear localization signal (NLS) and were predicted to be in the nucleus (Table 1).

3.2. Phylogenetic analysis

To investigate the phylogenetic relationships of the *SiCAMTA* gene family, an unrooted phylogenetic tree was constructed using the 88 CAMTA proteins of eight oilseed crops, *O. sativa*, and *A. thaliana* (**Figure 1; Supplementary Table S1**). The phylogenetic analysis showed that the CAMTA proteins were clustered into six groups (Group I-Group VI). Along with six *A. thaliana* CAMTA (AtCAMTA), all five SiCAMTA identified in the sesame genome were distributed in four groups, such as Group-II, Group-IV, Group-V, and Group-VI. Group-IV, with 25 CAMTAs, was the largest group, containing AtCAMTA4 orthologues, including SiCAMTA4a/4b. AtCAMTA3 orthologous proteins (18 CAMTAs) were clustered into group V, which included SiCAMTA3, while SiCAMTA5 present in group-II showed high sequence similarity with AtCAMTA5 and AtCAMTA6. Notably, group-VI, containing 20 CAMTAs, harbored AtCAMTA1 and AtCAMTA2 orthologues along with SiCAMTA2 (Fig. 1).

3.3. Domain composition of SiCAMTAs

To better understand the potential functions of SiCAMTAs, conserved domains were analyzed. SiCAMTA2 and SiCAMTA4a/4b contain conserved domains of a typical CAMTA protein (Fig. 2), while SiCAMTA3 and SiCAMTA5 were predicted to be non-TIG CAMTA. (Fig. 2). Moreover, CaMBD was identified in all five SiCAMTAs (Fig. 2). We identified the CaMBD conserved motif sequence as WXVX(2)LXKX (2)LRWRX[KR]X(3)[LF]X(2) in SiCAMTAs. This also showed that amino acid residues are highly conserved at certain positions, such as W(1)V(3) L(6)K(8)L(11)R(12)W(13)R(14) (Fig. 2). The motif scan identified NLS in all five SiCAMTAs, and it was placed within the CG-1 domain (Fig. 2). Moreover, two IQ domains were present in SiCAMTA4a/4b/5, while SiCAMTA2/3 had only one IQ domain.

3.4. Motif distribution and exon-intron structure of SiCAMTAs

The conserved motifs were predicted using MEME Suite to examine the structural features of SiCAMTAs. A total of 15 different motifs were found distributed throughout the SiCAMTAs protein sequences, with lengths ranging from 15 to 50 amino acids (**Figure 3A; Supplementary Table S3**). The number of motifs varied from 12 to 15 among five

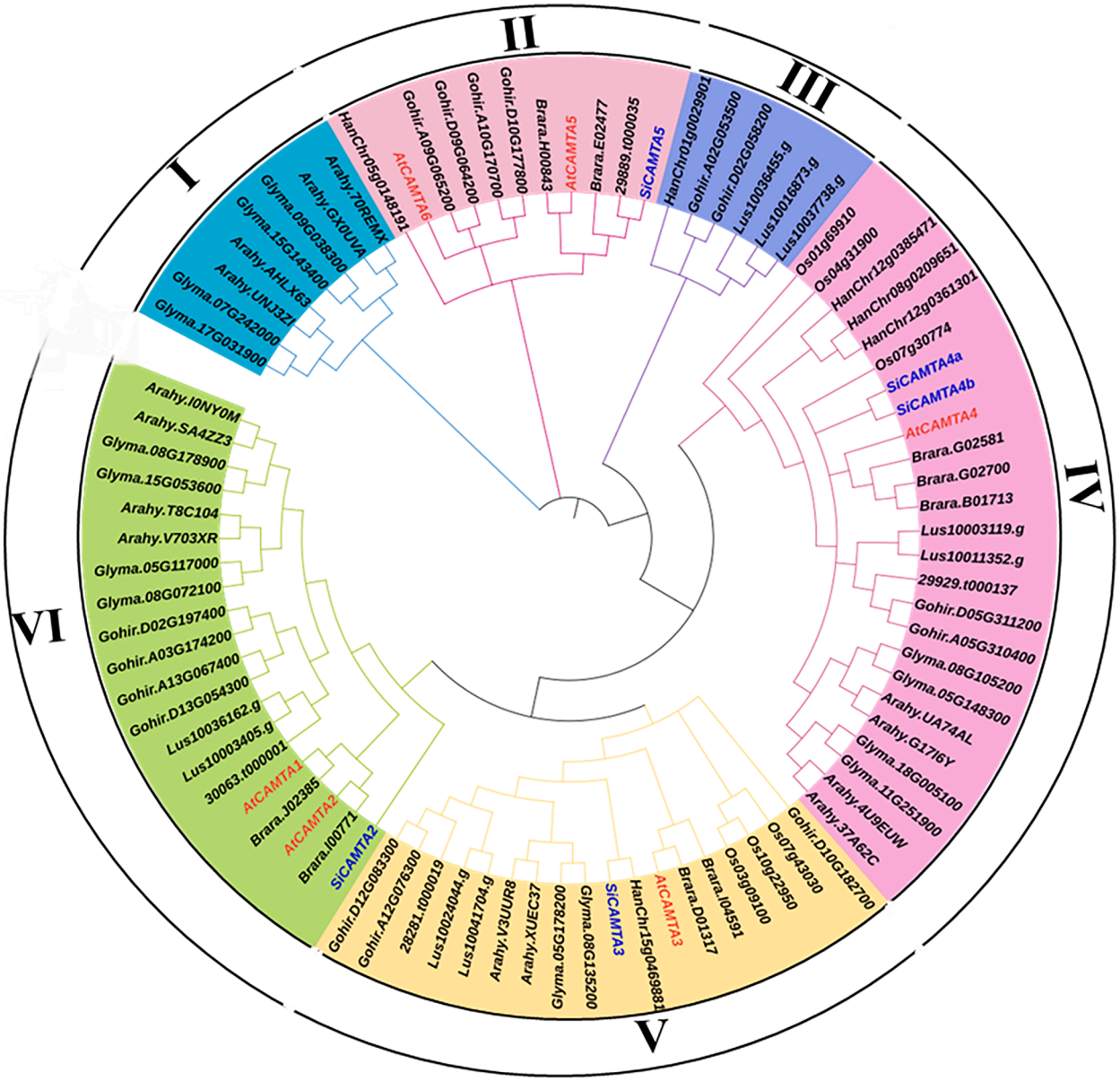

Fig. 1. Phylogenetic relationship of CAMTA proteins from *Arabidopsis*, rice and eight oilseed crops including *S. indicum*. The phylogenetic tree was constructed using MEGA-X by the neighbor-joining (NJ) method and 1000 bootstrap replicates. The blue and red color represent CAMTA proteins in *S. indicum* and *A. thaliana*, respectively. For details of the genes, refer to **Supplementary Table S1**.

SiCAMTA proteins. The motif 13 was characteristic of SiCAMTA4a/4b. Moreover, all five SiCAMTAs displayed motifs 4/1/11 and motif 10 which corresponded to the CG-1 domain and CaMBD while motif 12 was present specifically in SiCAMTA2 and SiCAMTA4a/4b overlapped with IPT/TIG domain (Fig. 3**A**). The structure and motif conservation support the results of the phylogenetic analysis. Further, the gene structure analysis showed that intron numbers contained in *SiCAMTA*s range from 10 (*SiCAMTA4a/4b)* to 12 *(SiCAMTA2/3/5)* (Fig. 3**B**). The intron phase pattern of '1102–0110–0200′ observed in *SiCAMTA3/5* and *SiCAMTA4a/4b* showed '1020–110–200′ and '1020–110–200′. However, *SiCAMTA2* showed a different pattern with '1102–0111–0200′.

3.5. Chromosomal distribution and synteny analysis of SiCAMTA genes

SiCAMTA4a/4b/3 were localized on Chr2, Chr8, and Chr13, respectively, while *SiCAMTA2/5* were co-localized on Chr10 (Fig. 4**A**). In addition, the collinearity relationships of *SiCAMTAs* within the *S. indicum cv. Baizhima* genome also showed a similar chromosomal distribution (Fig. 4**B**). In total, two segmental duplication events (*SiCAMTA2/3* and *SiCAMTA4a/4b*) were identified in the sesame genome. Fig. 4**B**. The Ka/Ks ratios of two segmentally duplicated *SiCAMTA* genes were 0.23 and 0.22, lower than 1 for *SiCAMTAs* (**Supplementary Table S4**). In addition, the duplicated events were estimated to have occurred approximately ~41 and ~135 million years ago for *SiCAMTA4a/4b* and *SiCAMTA2/3*, respectively, (**Supplementary Table S4**).

To further investigate the evolutionary relationship and functional insight of the *SiCAMTA* genes, we constructed comparative syntenic maps of *S. indicum* associated with *A. thaliana, B. rapa,* and *O. sativa* (Fig. 4**C**). The orthologs of all 5 *SiCAMTA* genes were present in *B. rapa*, while 4 *SiCAMTA* and only 2 *SiCAMTA* were present in *A. thaliana* and *O. sativa* across 4 chromosomes of sesame (**Supplementary Table S5**). Five *SiCAMTA* genes separately correspond to 8, 12, and 4 orthologous gene pairs in *A. thaliana B. rapa and O. sativa*, respectively. In sesame, chr10 exhibited the largest orthology (5 gene pairs), while one each of the orthologous gene pairs was detected on chr2, chr8, and chr13,

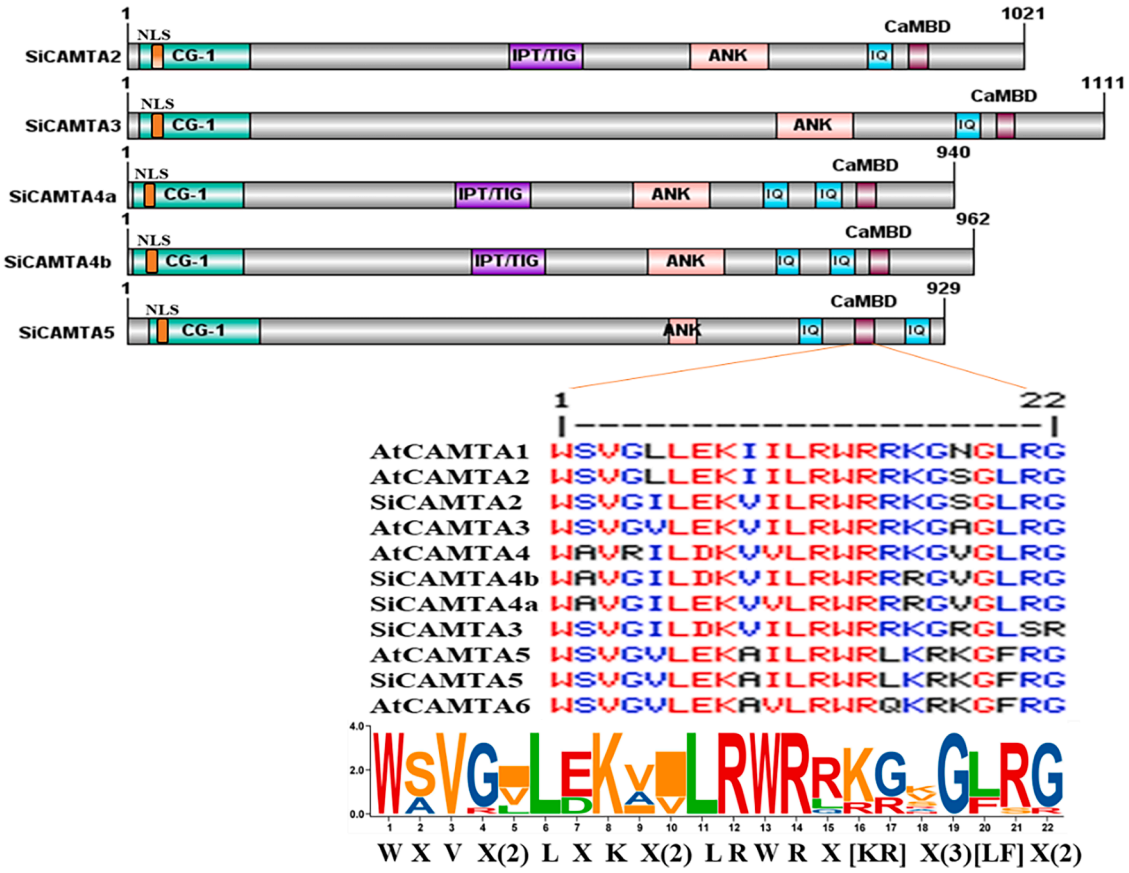

Fig. 2. Distribution of functional domains of CAMTA family of sesame (SiCAMTA). Schematic representation of functional domains of five SiCAMTA proteins, CaMBD sequence alignment and the CaMBD sequence logo of SiCAMTA proteins. CG-1, DNA binding domain; NLS, Nuclear localization signal; ANK, ankyrin repeats; IQ, Ca^{2+}-independent CaM-interacting domain; TIG/IPT, non-specific DNA binding domain; CaMBD, Ca^{2+}-dependent CaM binding domain. "X" stands for any amino acid, "()" represent the number of amino acids and "[]" denotes the amino acids allowed in this position of the CaMBD.

thereby leading to the identification of eight orthologous pairs from *A. thaliana*. Similarly, from *B. rapa*, the orthologous gene pairs identified were: chr10 of sesame had 4 orthologous gene pairs, while chr2, chr8 and chr13 were found to have 2 orthologous gene pairs. However, orthologous gene pairs from *O. sativa* were found in sesame chr8 (1 gene pairs) and chr13 (3 gene pairs).

3.6. Cis-regulatory elements in the promoter of SiCAMTA genes

Cis-acting regulatory elements (CAREs) are specific motifs present in the promoter regions of a gene. Using the PlantCARE database, a total of 50 CAREs were identified in the promoter region of five *SiCAMTA* genes (**Supplementary Table S6**). The type and number of CAREs differed in the promoter region of the five *SiCAMTA* genes (Fig. 5**A**). The highest number of total CARE binding sites were detected in the *SiCAMTA5* gene promoter (48 sites) and the least in *SiCAMTA4b* (31 sites) (Fig. 5**B**). Based on their functional relevance, the identified CAREs were categorized into four groups: abiotic and biotic stress, phytohormone, light, and growth-development responsive (Figs. 5**A and B**). The binding sites for the stress response-associated CAREs were predominant in all five *SiCAMTA* promoters, with the highest for *SiCAMTA4a* (26) (Fig. 5**B**). Further, stress-responsive CAREs like ARE (anaerobic response), MYB, MBS, and MYC were identified in most *SiCAMTA* genes (Figs. 5**A and C**). In the phytohormone-response groups, sites for ABRE (abscisic acid-responsive), ERE (ethylene-responsive), and TGA (auxin-responsive) were predominantly observed in *SiCAMTA2* and *SiCAMTA5* genes. Gibberellin-responsive motifs such as P-box and GARE were found in *SiCAMTA3/4a/5*. CGTCA and TGACG-motif of methyl jasmonate-response were present in *SiCAMTA3* and *SiCAMTA4b* (Figs. 5**A and C**).

3.7. RNA-seq. expression profile of SiCAMTAs during drought and waterlogging stress

To investigate the functional significance of *SiCAMTA* genes, we obtained the expression profiles of five significantly differentially expressed (p-adjusted value $\leq$0.05) *SiCAMTA* genes during waterlogging and drought stress. The expression profile of the *SiCAMTA* gene was obtained for the -tolerant genotype (ZZM0635: DT) and the drought-sensitive genotype (ZZM4782:DS) at control (d0) and three stages (d1, d2, and d3) of drought stress treatment (Dossa et al., 2019). The differential expression (log2FC) in the DT genotype in comparison to the DS genotype is depicted in Fig. 6**A**. All *SiCAMTAs* exhibited upregulation in the DT genotype at d3 treatment, except *SiCAMTA3*, which showed the highest expression at the control condition in the DT genotype (Fig. 6**A**). *SiCAMTA4a* and *SiCAMTA4b* demonstrated increased expression at d1 and d2 conditions in the DT genotype (Fig. 6**A**).

The expression of *SiCAMTA2* and *SiCAMTA5* was recorded as highest in the DT genotype at d3 drought treatment. Upon comparison between the control and treatment groups, consistent *SiCAMTA3* expression was revealed across all time points. *SiCAMTA4b* consistently decreased from d1 to d3 in both DS and DT genotypes (Fig. 6**B**). *SiCAMTA4a, SiCAMTA2*, and *SiCAMTA5* showed increased expression, mainly noticeable in the DT genotype at the d2 and d3 stages (Fig. 6**B**).

The expression profiles of five *SiCAMTA* genes were obtained in waterlogging-tolerant (ZZM2541: WT) and waterlogging-sensitive (Ezhi No. 2: WS) sesame genotypes at four stages (0, 12, 24, and 48 h) of waterlogging stress (Wang et al., 2021). The *SiCAMTA* genes exhibited different expression patterns in response to waterlogging stress (Figs. 6**C**

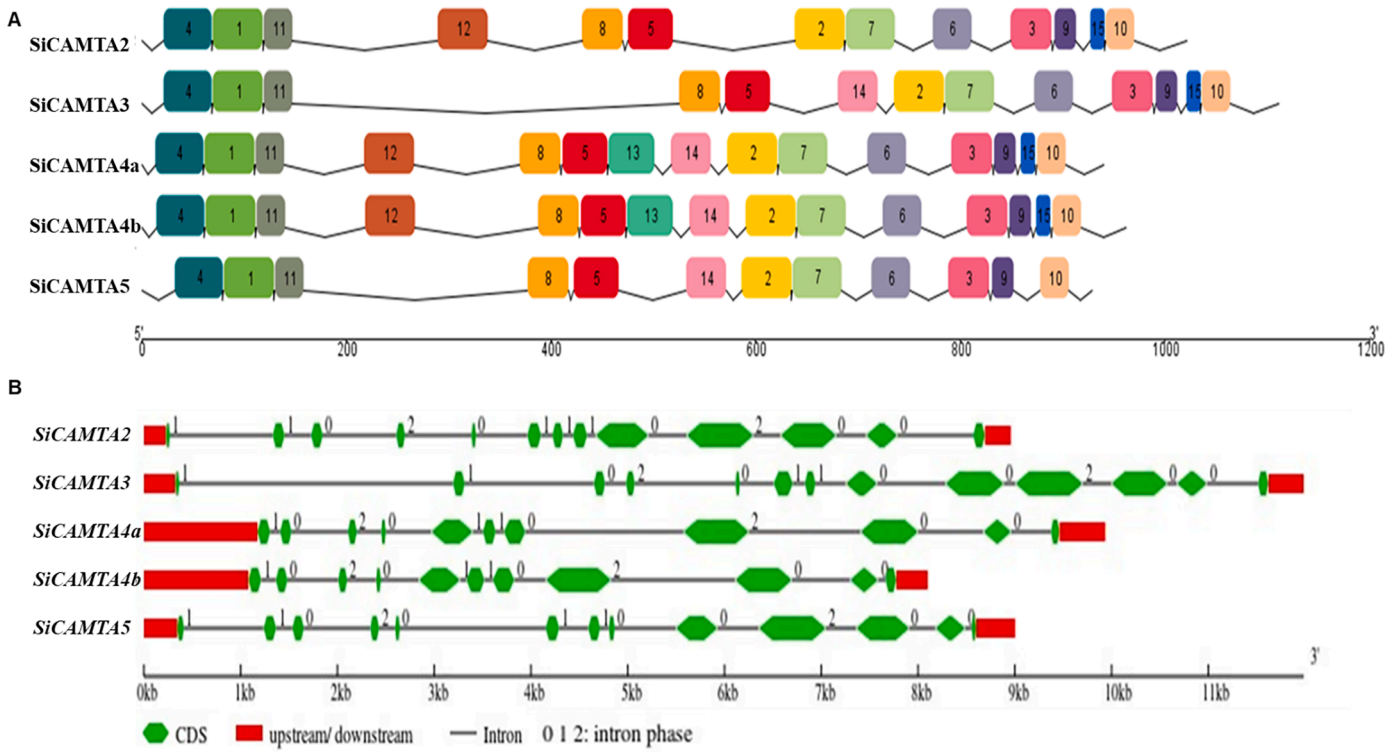

Fig. 3. Distributions of motifs and exon-intron structure of *SiCAMTA* genes. (A) Motif composition of the SiCAMTA family in sesame. Conserved motifs are indicated by colored boxes with their respective motif numbers. Further details on each motif are provided in **Supplementary Table S3. (B)** The exon-intron structure of *SiCAMTA* genes. Red boxes represent UTR regions, black lines represent introns and green boxes represent exons. The numbers (0,1,2) denote intron phase.

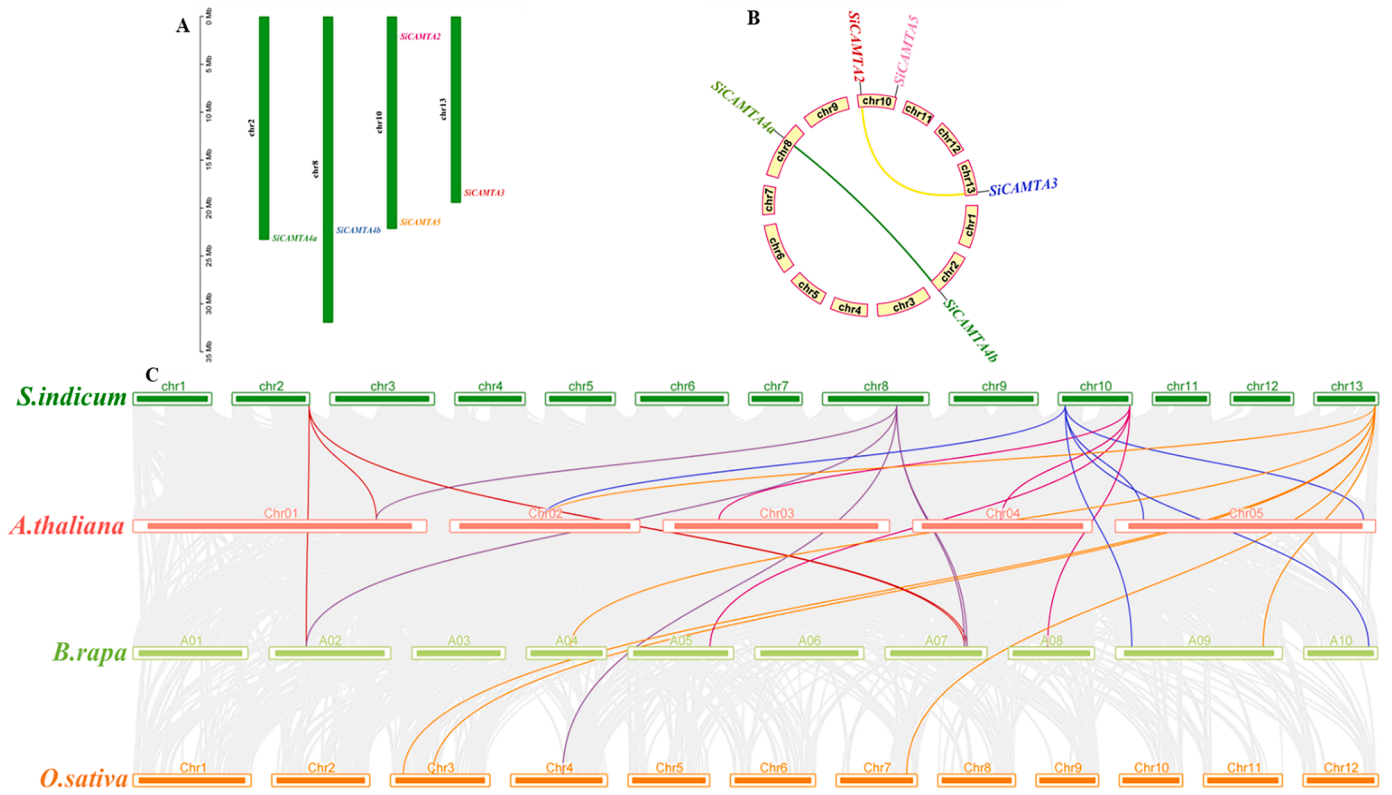

Fig. 4. Chromosomal localization, collinearity, and synteny analysis of *SiCAMTA* genes. (A) The chromosomal localization of *SiCAMTAs* in *S. indicum cv. Baizhima* genome. **(B)** Collinearity analysis of *SiCAMTAs* within *S. indicum cv. Baizhima* genome. **(C)** The interspecies multiple synteny analysis of *SiCAMTA* genes from sesame, *A. thaliana, B. rapa* and *O. sativa.* Grey lines in the background indicated the collinear blocks, while coloured (red, pink, purple, blue and orange) lines highlight the syntenic *SiCAMTA* gene pairs among four species.

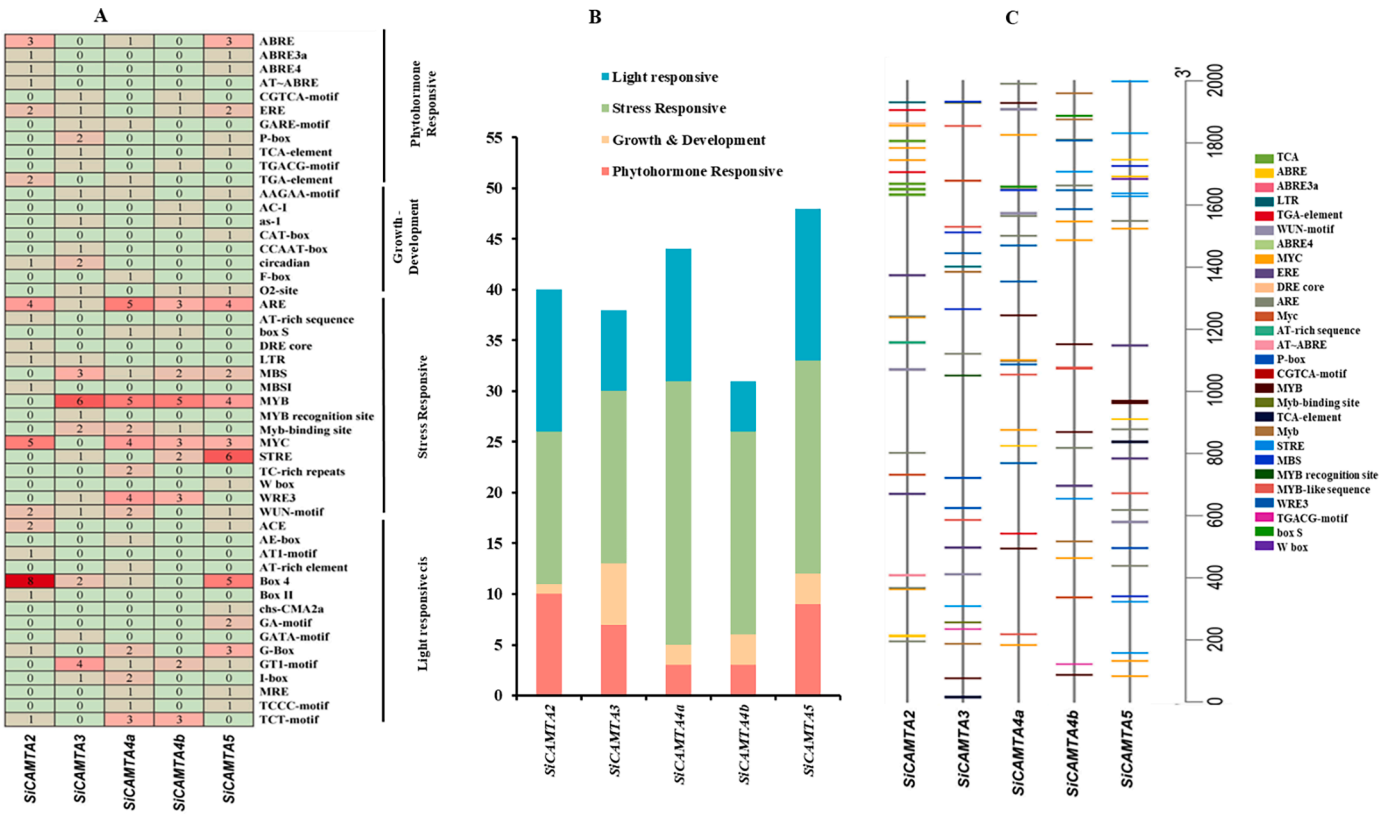

Fig. 5. Predicted cis-acting regulatory elements (CAREs) in putative promoters (2000 bp upstream of transcriptional start site) sequences of *SiCAMTA* genes. (A) Distribution of different CAREs in *SiCAMTAs* putative promoters. Counts of each CAREs are shown in the column diagram. Based on the functional annotation, the CAREs were classified into four major categories: light responsive-, stress responsive, phytohormone, and growth and development responsive. **(B)** Sum of the CAREs in each category for *SiCAMTA* gene. **(C)** Stress and phytohormone-response related CAREs in the promoter of *SiCAMTA* genes.

and D). All *SiCAMTAs* exhibited up-regulation in the WT genotype at 48 h of waterlogging stress, except *SiCAMTA4b* (Fig. 6**C**). *SiCAMTA2, SiCAMTA3*, and *SiCAMTA5* were upregulated in WT at 24 h and 48 h while down-regulated or unchanged at 0 h and 12 h of waterlogging stress (Fig. 6**C**). In contrast, *SiCAMTA4b* recorded its highest expression at 0 h and its lowest expression levels at 12 h and 48 h in WT (Fig. 6**C**). Further, in comparison to the control, *SiCAMTA4b* and *SiCAMTA2* were constantly downregulated at 12 h, 24 h, and 48 h in both WS and WT genotypes, with lower expression in WT at each time point. However, in contrast, *SiCAMTA4a, SiCMATA3*, and *SiCAMTA5* were upregulated in both WS and WT at each time point, with the lowest at 24 h of waterlogging stress (Fig. 6**D**).

3.8. Identification of SiCAMTA-regulated genes in sesame genome and their expression profiles during drought and waterlogging stress

CAMTA has been identified to exhibit a distinctive binding affinity to the CGCG box within the promoter regions of its target genes (Yang and Poovaiah, 2002). In this study, a total of 1202 genes were identified as potential targets of five *SiCAMTA genes* (**Supplementary Table S7**). The promoters of stress responsive genes such as *late embryogenesis abundant At1g64065-like* (*LEA*), *polyphenol oxidase I*, chloroplastic-like (*PPO1*), *respiratory burst oxidase homolog protein C-like* (*RBOH—C*), *aquaporin PIP1–2* (*PIP1–2*), *senescence-associated protein* (*SAP*), *cell wall integrity/-stress response component-like protein* (*WSC*), *acidic mammalian chitinase-like, E3 ubiquitin- ligase RHA2B-like* (*E3 RHA2b*), *gibberellin 3-beta-dioxygenase 1-like* (*GA3OX1*), *auxin response factor 17* (*ARF 17*), and *protein DMR6-like OXYGENASE 1* (*DLO1*) were enriched with *SiCAMTA* recognition cis-element (**Supplementary Table S6**).

3.9. RNA-seq. based expression profile of SiCAMTA target genes during drought and waterlogging stress

The expression profile of the *SiCAMTA* target gene in response to drought and waterlogging stress was obtained by comparing transcriptome data of sensitive and tolerant genotypes at different stages of drought and waterlogging stress treatment conditions (Dossa et al., 2019; Wang et al., 2021). From a total of 1202 *SiCAMTA* target genes, 240 and 224 genes were significantly (p-adjusted value $\leq$0.05) differentially expressed during waterlogging and drought stress, respectively (**Supplementary Table S8**). Moreover, 47 and 27 genes were found to have log2FC>2 differential expression during drought and waterlogging stress in sesame, respectively (**Supplementary Table S8**). During drought stress, the expressions of most of the selected *SiCAMTA* target genes were either up-regulated or unchanged at initial stage of drought treatment (d1 and d2) and then down-regulated with prolonged drought treatment (d3), except for *E3-RHA2B*, which showed up-regulation in the -tolerant genotype (Figs. 7**A and B**). In particular, *ARF17* and *DLO* at d1, whereas *SAP* and *LEA* at d2, were significantly up-regulated in drought-tolerant genotypes. However, during waterlogging, in contrast to drought, the expressions of most of the selected *SiCAMTA* target genes were significantly up-regulated in the tolerant genotype except *SAP*, which showed down-regulation during 48 h of waterlogging stress (Figs. 7**C and D**).

3.10. Interaction network of SiCAMTA proteins

A protein-protein interaction network (PPI) is a helpful preface to exploring the biological functions of unknown proteins. Results showed that the five SiCAMTA proteins corresponded to four *A. thaliana* homologs (**Figure 8; Supplementary Table S9**). The PPI network revealed

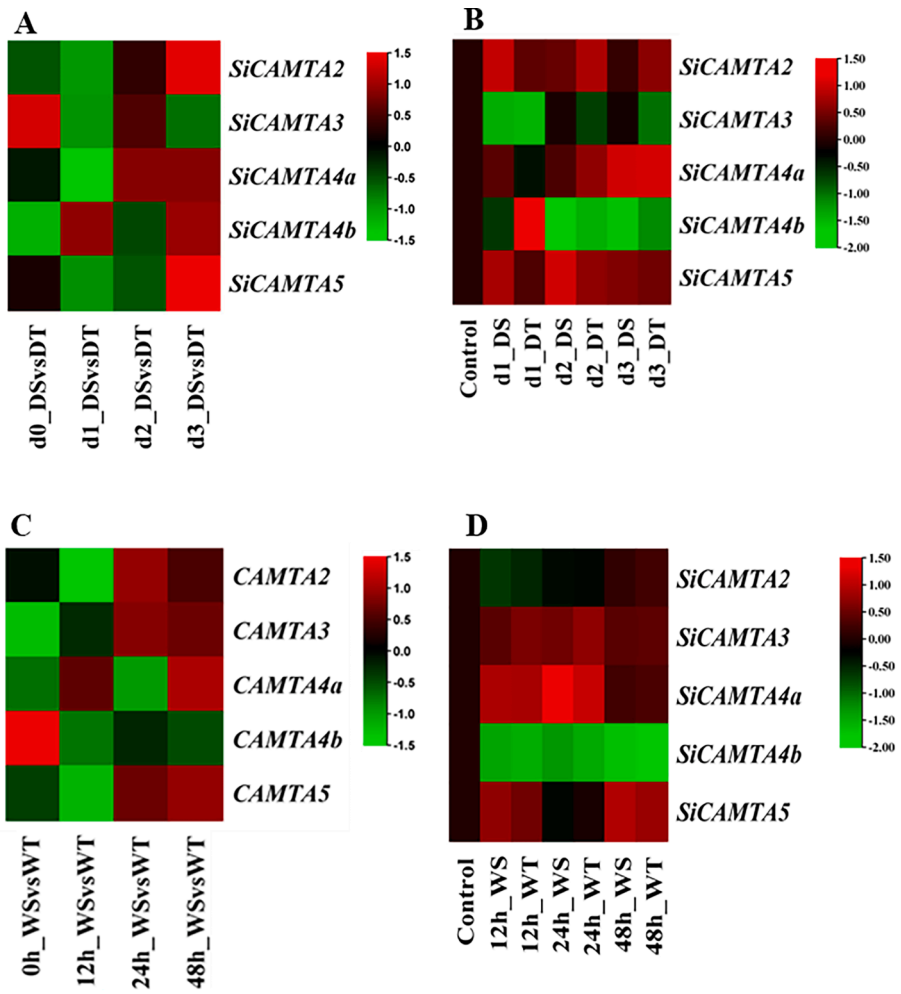

Fig. 6. RNA-seq. expression profile of *SiCAMTAs* during drought (A and **B) and waterlogging stresses (C** and **D)**. (**A**) Expression pattern of *SiCAMTAs* in drought tolerant (DT; ZZM0635) in comparison with drought sensitive (DS; ZZM4782) genotype at different stage of drought stress. (**B**) Expression patterns of *SiCAMTAs* in drought tolerant (DT; ZZM0635) and drought sensitive (DS; ZZM4782) genotype at different stage of drought stress, d0 was set as control. (**C**) Expression profiles of *SiCAMTAs* at different stage of waterlogging stress in waterlogging tolerant (WT; ZZM2541) in comparison with waterlogging sensitive (WS; Ezhi No. 2) genotype. (**D**) Expression profile of *SiCAMTAs* at different stage of waterlogging stress in waterlogging tolerant (WT; ZZM2541) and waterlogging sensitive (WS; Ezhi No. 2) genotypes, 0 h was set as control. Log2FC values of the relative expression levels of ten *SiCAMTA* target genes under drought and waterlogging stresses were used to create the heat maps. Changes in gene expression are shown in color as the scale. Red color stands for high expression and the green color represents low expression.

various interactions of SiCAMTA with other proteins, including transcription factors, calmodulin binding proteins, calcium-related protein kinases, and functional proteins such as EDS1, GH3.12, and ICS1 **(Figure 8; Supplementary Table S9)**. The protein Q5XV94_ARATH, a NEFA-interacting nuclear protein, strongly interacted with all SiCAMTA proteins. Furthermore, DREB1C, a key regulator of cold tolerance, was found to interact with all SiCAMTAs except SiCAMTA2. The direct interaction of SiCAMTA4 with RD29A, COR47, and COR15A highlights its role in the abiotic stress response.

3.11. qRT-PCR based expression profile of SiCAMTA and target genes during drought and waterlogging stress

The RNA-seq expression profile of five *SiCAMTA* and ten *SiCAMTA* target genes were validated in drought-sensitive (GT-10-DS-1, Thilak-DS-2), drought-tolerant (IC129772-DT1, EC350648-DT2), waterlogging-sensitive (IC129289: WS1, IC131542: WS2), and waterlogging-tolerant (EC334977: WT1, EC334970: WT2) sesame accessions at 0 h and 48 h of drought and waterlogging stress using qRT-PCR (Fig. 9). The *SiCAMTA2/5*, were significantly upregulated in both DT1 and DT2 during drought stress at 48 h, consistent with RNA-seq profile at d3. Similarly, *SiCAMTA3/4b* exhibited comparable expression at d2 (Fig. 9**A and C**). Additionally, *SiCAMTA2/34a/5* were significantly upregulated in tolerant genotype at 48 h of waterlogging stress, aligning with RNA-seq. data (Fig. 9**B and D**). The qPCR expression showed that *E3-RHA2B* was significantly up-regulated while *ARF17* was down-regulated in DT2 genotype at 48 h of drought treatment, corresponding with RNA seq expression profile at d3. Furthermore, the qPCR expression profile of *RBOH—C*, and *PIP1–2* were consistent at d2 whereas *PPO1* and *WSC* showed opposite expression patterns (Fig. 9**E and G**). In addition, similar to RNA-seq, qPCR analysis had showed that *GA3OX1,* and *DLO* were significantly up-regulated in tolerant genotype at 48 h of waterlogging stress while the relative expression of *ROBH—C, LEA, PIP1–2, PPO1* corresponds with expressions at 12 h and 24 h (Fig. 9**F and H**).

4. Discussion

With the availability of sesame genome datasets and bioinformatics tools, sesame is receiving growing attention in the genome-wide gene identification and characterization of multiple gene copies in each gene family. Sesame is an important oilseed crop, and its growth and productivity are severely affected by abiotic stresses. Sesame plants are

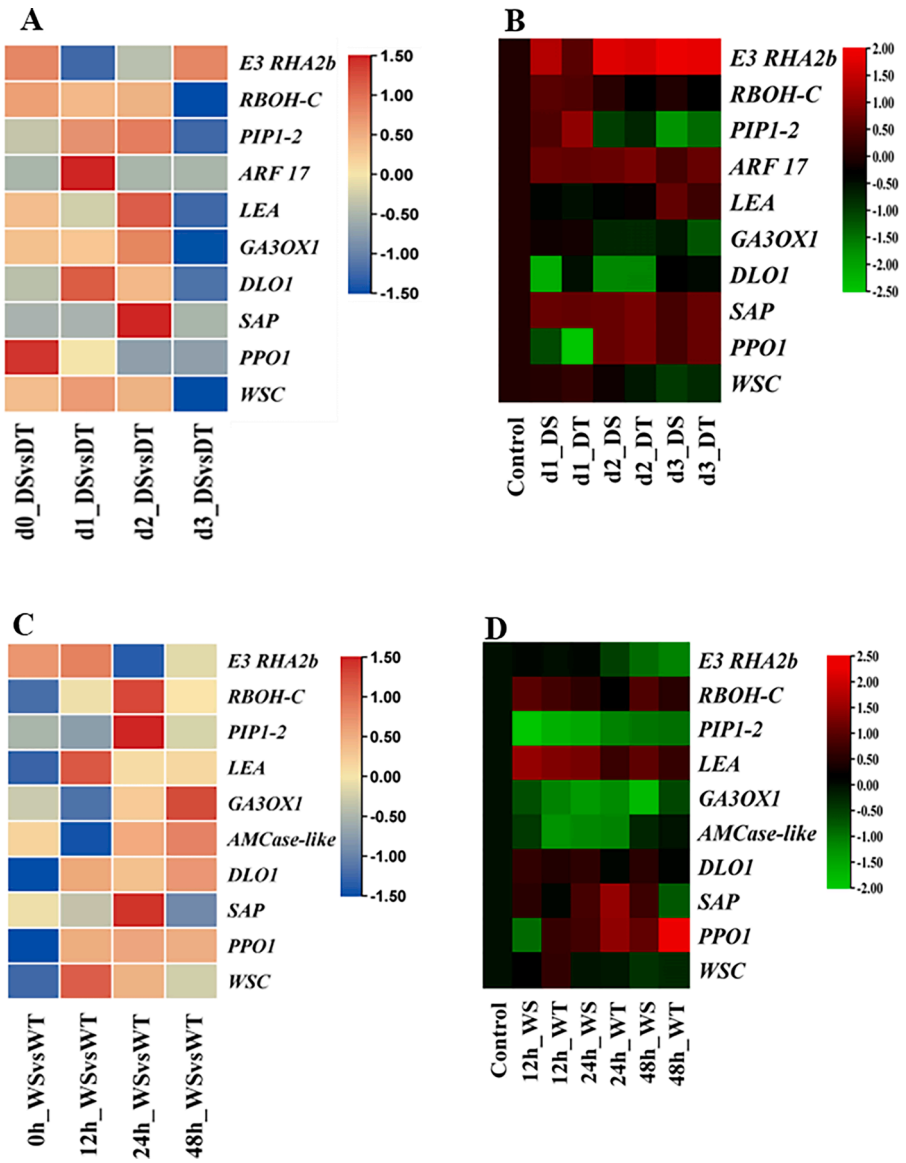

Fig. 7. RNA-seq. expression profile of ten *SiCAMTA* target gene during drought (A and B) and waterlogging stress (C and D). (**A**) Expression pattern of *SiCAMTA* target genes in drought tolerant (DT; ZZM0635) in comparison with drought sensitive (DS; ZZM4782) genotype at different stage of drought stress. (**B**) Expression pattern of *SiCAMTA* target genes in drought tolerant (DT; ZZM0635) and drought sensitive (DS; ZZM4782) genotype at different stage of drought stress, d0 was set as control. (**C**) Expression profile of *SiCAMTA* target genes at different stage of waterlogging stress in waterlogging tolerant (WT; ZZM2541) in comparison with waterlogging sensitive (WS; Ezhi No. 2) genotype. (**D**) Expression profile of *SiCAMTA* target genes at different stage of waterlogging stress in waterlogging tolerant (WT; ZZM2541) and waterlogging sensitive (WS; Ezhi No. 2) genotypes, 0 h was set as control. Log2FC values of the relative expression levels of ten *SiCAMTA* target genes under drought stress and waterlogging stress were used to create the heat maps. Changes in gene expression are shown in color as the scale. Red color stands for high expression and the green and blue color represents low expression.

often subjected to multiple abiotic stresses simultaneously during their cultivation (Anee et al., 2019; Su et al., 2022; Wang et al., 2021). The *CAMTA* TF genes were well characterized, but only in model plant species. The implication of *CAMTA* genes in abiotic stresses was less studied in sesame. We undertook a comprehensive genome-wide identification and expression analysis to establish the role of *SiCAMTA* TFs in abiotic stress tolerance, particularly drought and waterlogging in sesame.

In this study, the five *SiCAMTA* genes identified in the sesame genome were consistent with the 6–8 *CAMTA* gene numbers identified in other plant species such as *A. thaliana* (6), *B. campestris* ssp. *chinensis* (8), and *O. sativa* (7) (Hu et al., 2015; Zhang et al., 2019; Gain et al., 2022). The length of the identified SiCAMTA protein (929 to 1111 amino acids) was similar to the CAMTA protein of other plants (Table 1). All five SiCAMTAs were identified as hydrophilic, unstable, and predicted to be located in the nucleus (Table 1).

The phylogenetic analysis divided five *SiCAMTA* genes into four groups, along with *AtCAMTAs* (Fig. 1). The genes within the same group shared similar gene structures in terms of either exon length or intron number. Therefore, we speculated that the SiCAMTAs in the same group may have similar functions, and this feature was similar to that previously reported in other species. In particular, SiCAMTA2 clustered with AtCAMTA2 would potentially associate with drought and osmotic stress tolerance functions, as known for the genes of *A. thaliana AtCAMTA1 and AtCAMTA2* (Pandey et al., 2013; Shen et al., 2015). Similarly, SiCAMTA3 clustered with AtCAMTA3 reported to be involved in cold response, pathogen defense response, and resistance to insect herbivory (Yang et al., 2015; Pant et al., 2018). The involvement of these *SiCAMTA* genes, on a homology basis, shows potential characteristic features to be involved in the regulation of a cascade of genes during biotic and abiotic

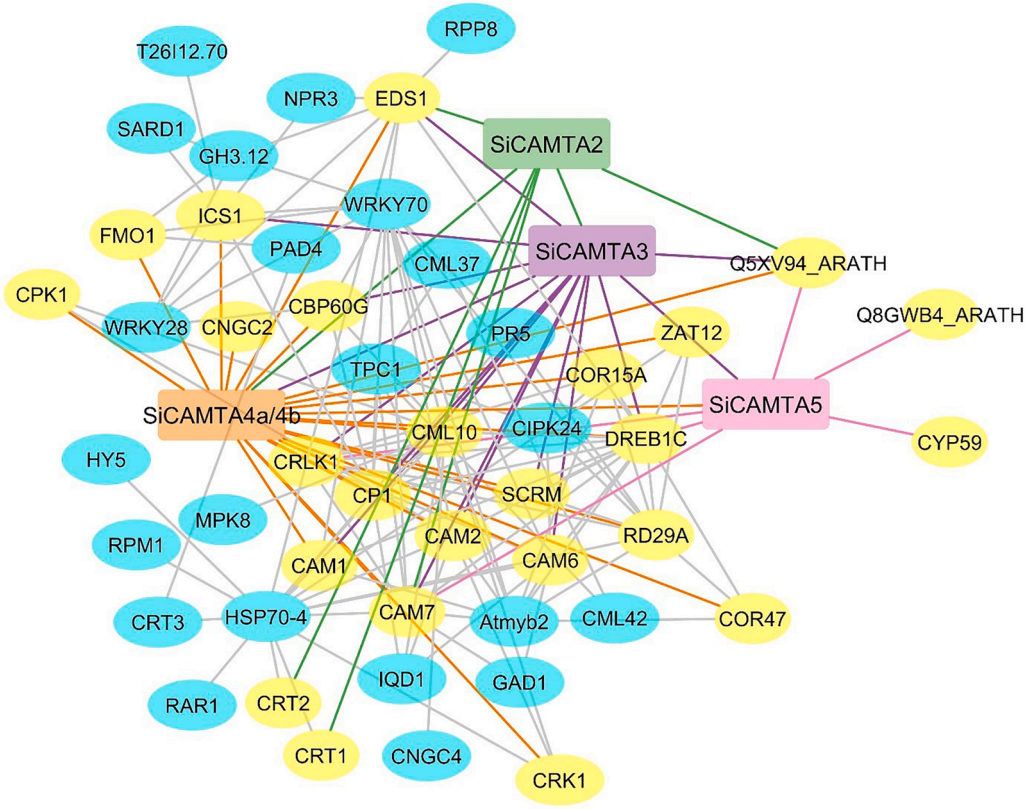

Fig. 8. Interaction network of five *SiCAMTA* genes and related genes in *A. thaliana*. PPI network of SiCAMTA protein orthologs was predicted in STRING database (v.12.0) and visualized using the Cytoscape tool (v. 3.10.1). Each node represents a protein, with edges signify interactions. The thickness of the lines reflects the strength of the interactions. Direct interactions of SiCAMTA with other proteins are highlighted in yellow nodes while the cyan circle color depicted the indirect interactions.

stress responses for imparting tolerance.

CAMTAs are known as the largest and best-characterized CaM-binding TFs (Iqbal et al., 2020). The typical CAMTA is composed of highly conserved functional domains across the species, including CG-1, NLS, TIG/IPT, ANK, IQ, and CaMBD domains (Finkler et al., 2007). In our study, five SiCAMTAs were categorized as TIG (SiCAMTA2, SiCAMTA4a, and SiCAMTA4b) and non-TIG-type CAMTAs (SiCAMTA3, and SiCAMTA5) (Fig. 2). It was also reported that the non-TIG/IPT domain of the CAMTA protein appeared in newly evolved flowering land plants, indicating these two SiCAMTA proteins emerged after the divergence of flowering plants (Rahman et al., 2016; Pant et al., 2018; Büyük et al., 2019). The consensus sequence [WXVX(2)LXKX(2)LRWRX [KR]X(3)[LF]X(2)] of SiCAMTAs CaMBD was conserved from *A. thaliana*, cotton, and tea plants (Fig. 2), (Bouché et al., 2002; Pant et al., 2018; Zhou et al., 2022). The presence of both IQ and CaMBD domains in all five identified SiCAMTA proteins signifies that they can interact with calmodulin in Ca^{2+}-independent and Ca^{2+}-dependent pathways, respectively (Fig. 2). The variation in IQ domain number among five SiCAMTAs (Fig. 2) suggests their different binding specificities for CaM or CaM-like proteins.

The intron phases are strongly linked to the evolution of eukaryotic genomes and are conserved throughout evolution. The presence of introns can occur in three phases: phase-0, 1, and 2, with phase-0 introns being the most common (Long et al., 1995; Nguyen et al., 2006). The gene structure analysis showed that the 10–12 introns of *SiCAMTA* genes were consistent with the 12 introns found in the *CAMTA* gene of higher plants. In the eukaryotic genome, the existence of introns is believed to be important for gene expression regulation and new functional protein generation (Wu et al., 2019). Similar to previous studies, our results also showed that the lengths of the exons corresponding to intron phase pattern '0200' were significantly longer than those corresponding to other intron phases (Fig. 3**B**). Chromosomal localizations of five *SiCAMTA* genes showed their uneven distribution on four chromosomes. Segmental and tandem duplication are the major driving forces for the expansion of gene family members. In this study, we found that the *SiCAMTA* gene family expands *via* segmental duplication events under purifying selection. Segmental duplications and purifying selection have been widely observed for *CAMTA* family genes in other plants like tea plants (Li et al., 2022) and bananas (Meer et al., 2019).

The comparative synteny analysis of sesame with two dicot (*A. thaliana, B. rapa*) and one monocot (*O. sativa*) species further highlights the potential evolutionary mechanisms of the *SiCAMTA* gene family. *A. thaliana* and *Brassica* belong to the Brassicaceae family and share a common ancestor. Around 17 to 18 MYA, *Brassica* diverged from *A. thaliana* (Yang et al., 2006). *B. rapa* has undergone additional genome triplication (13 to 17 MYA) just after diverging from *A. thaliana* (Blanc et al., 2003; Bowers et al., 2003). However, the divergence time of monocot-dicot was estimated at 200 MYA (Wolfe et al., 1989). The number of *SiCAMTA* orthologous pairs was higher in dicot (12 in *B. rapa* and 8 in *A. thaliana*) species than that of monocot (4 in *O. sativa*) (**Figure 4; Supplementary Table S5**), which was consistent with the closer evolutionary distance between sesame, *B. rapa,* and *A. thaliana* than *O. sativa*. Moreover, the higher number of ortholog pairs in *SiCAMTA-BrCAMTA* than *SiCAMTA-AtCAMTA* might be the result of an additional genome triplication event in the *B. rapa* genome after divergence from *A. thaliana* (Yang et al., 2006). In this study, two segmental duplication events for the *SiCAMTA* (*SiCAMTA2/SiCAMTA3* at ~135 MYA and *SiCAMTA4a/SiCAMTA4b* at ~41 MYA) gene was observed. Further, the presence of orthologs for all 5 *SiCAMTA* genes in *B. rapa*, 4 *SiCAMTA* in *A. thaliana*, and only 2 *SiCAMTA* in *O. sativa* further supported the evolutionary distance and duplication event (**Supplementary Table S5**). Similarly, in comparative synteny analysis, different

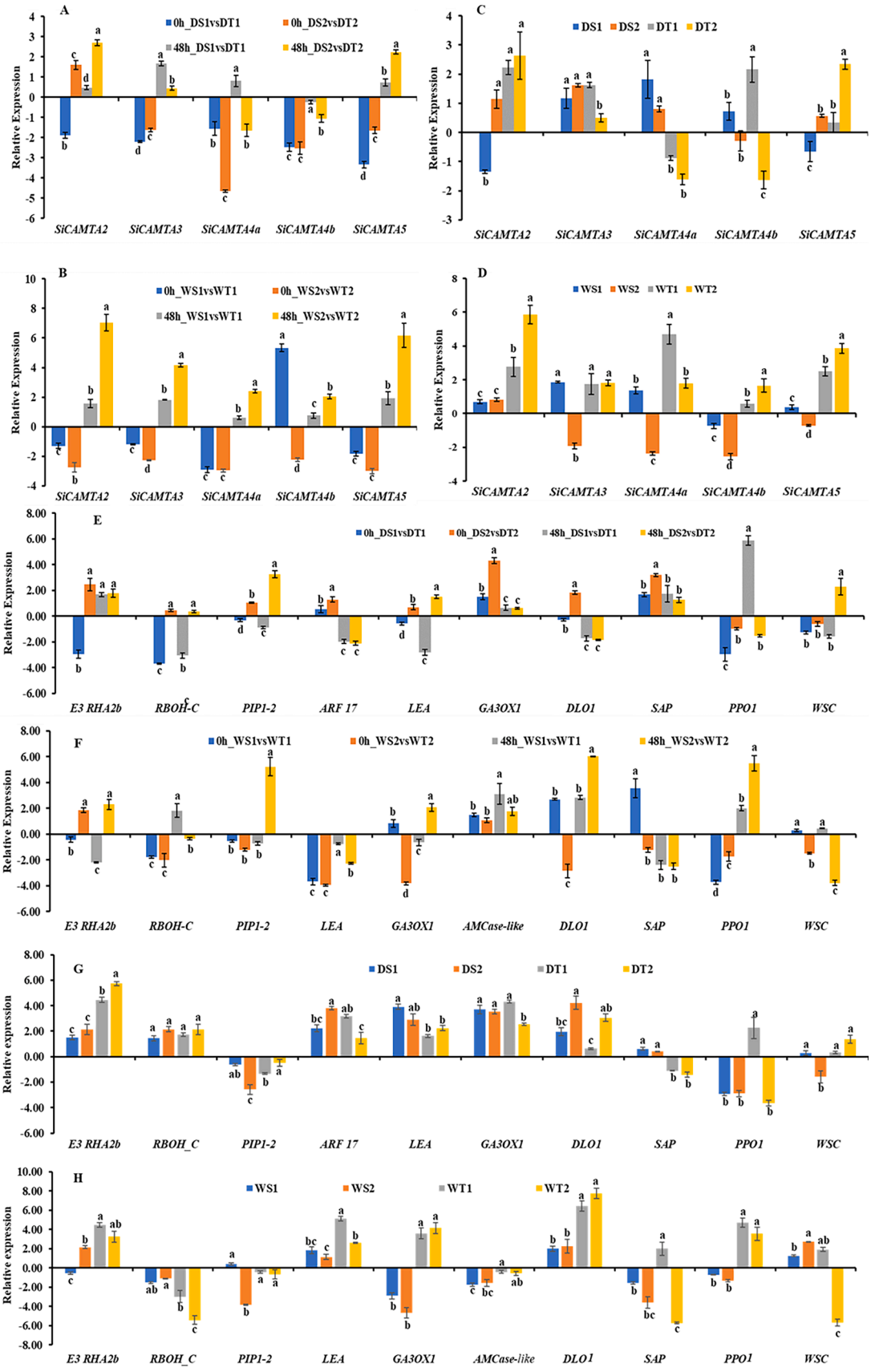

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Fig. 9. qRT-PCR based relative expression of *SiCAMTA* (A-D) and target genes (E-H) during drought and waterlogging stress. The relative expression of *SiCAMTAs* in two drought (DT1:IC129772; DT2:EC350648) and two waterlogging tolerant sesame accessions (WT1:EC334977; WT2:EC334970) in comparison with drought sensitive (DS1:GT-10 and DS2:Thilak) and waterlogging sensitive (WS1:IC129289, and WS2:IC131542) sesame accessions at 0 hrs and 48 hrs drought and waterlogging stress, respectively (**A** and **B**). The relative expression of *SiCAMTAs* in drought and waterlogging tolerant as well as drought and waterlogging sensitive sesame accessions at 48 hrs of drought and waterlogging stress, 0 hrs was set as control (**C** and **D**). The relative expression of *SiCAMTAs* target genes in two drought and two waterlogging tolerant sesame accessions in comparison with drought and waterlogging sensitive accessions at 0 hrs and 48 hrs drought and waterlogging stress, respectively (**E** and **F**). The relative expression of *SiCAMTA* target genes in drought and waterlogging tolerant as well as drought and waterlogging sensitive sesame accessions at 48 hrs of drought and waterlogging stress, 0 hrs was set as control (**G** and **H**). Each graph represents the average ± SE from three independent technical replicates, obtained from pooled single biological sample from two plants. Statistical analysis was conducted using one-way ANOVA and followed by Duncan's range test in SPSS 14.0. Different lowercase letters denote significant differences in gene expression level ($p \leq 0.05$).

numbers of ortholog gene pairs have been reported for the *CAMTA* gene family in tea plants (Li et al., 2022).

To elucidate the potential regulatory roles of *SiCAMTAs* in stress response in sesame, we studied the distribution and frequency of CAREs. The promoters of *SiCAMTA* genes have also been found to contain CAREs that are known to modulate gene expression in response to various stresses. Among abiotic stress-responsive CAREs, MYC and MYB binding sites were present in all *SiCAMTA* promoters. They have been reported to play an important role in drought-inducible expression, indicating that *SiCAMTA* expression is associated with abiotic stress (Smita et al., 2015). The invariable presence of certain important abiotic stress responsive (STRE, ARE, DRE, LTR) and phytohormone responsive (ABRE, ABRE3a, ABRE4, ERE, P-box, TGA-element) CAREs in the promoter region of *SiCAMTAs* strengthened their involvement in abiotic stress responses (Fig. 5**A**). Conceivably, the occurrence of these motifs in the promoter regions of *SiCAMTAs* is indicative of plausible molecular regulation, which can be further validated through stress experiments.

CAREs are essential for gene expression, and their numbers are correlated with gene expression intensity (Peng et al., 2011). Among the identified *SiCAMTAs*, the promotors *SiCAMTA2* and *SiCAMTA5* contain

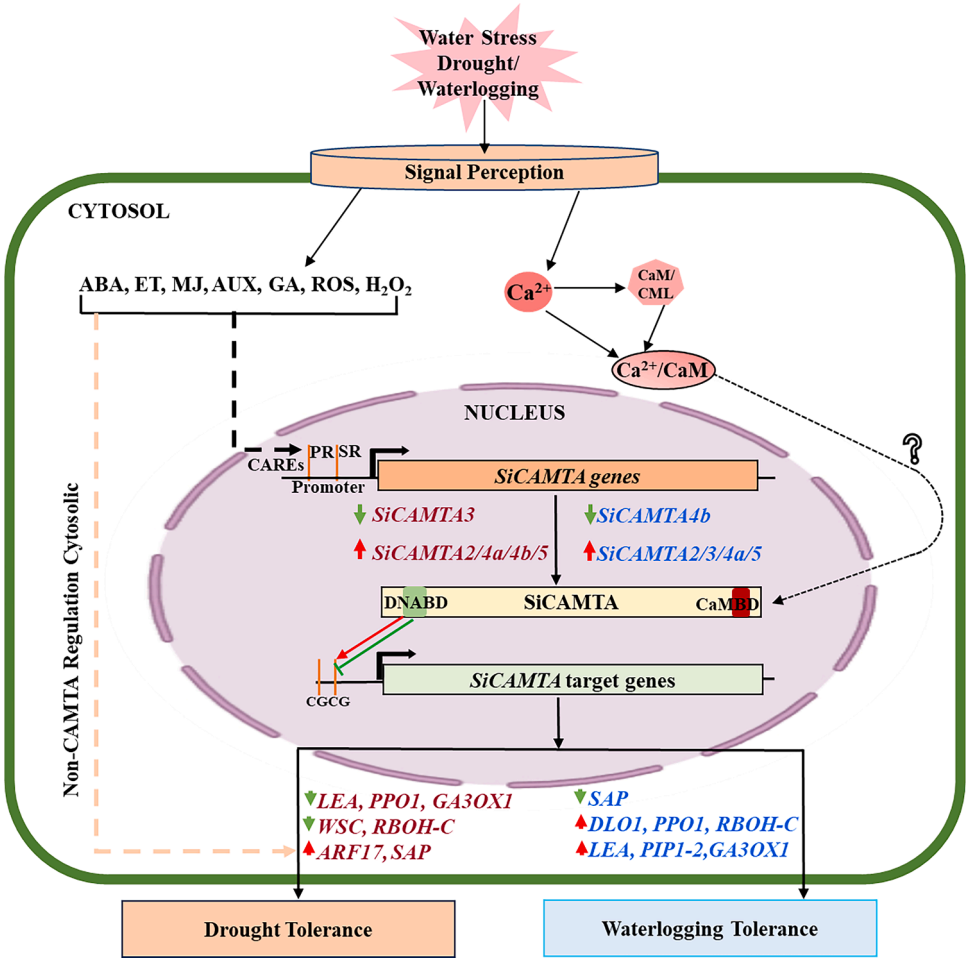

Fig. 10. The schematic illustration of the involvement of *SiCAMTA* and their target genes in drought and waterlogging tolerance in sesame. *SiCAMTAs* respond to variety of water stress signals through PR (Phytohormone responsive) and SR (Stress responsive) CAREs at promoter region and regulate the target gene expressions by recognizing the CGCG elements. Maroon and Blue coloured *SiCAMTA* and target genes represents regulation during drought and waterlogging stress, respectively. Red and green colour arrow represent up- and down regulation of *SiCAMTA* and target genes. The ABA, Abscisic Acid; ET, ethylene; MJ, methyl jasmonate; AUX, Auxin; GA; Gibberellic Acid; ROS, Reactive oxygen species; H_2O_2, Hydrogen peroxide, CaM/cis-CML, Calmodulin/Calmodulin-like; PR, Phytohormone responsive CAREs; SR, Stress responsive CAREs; DNABD, DNA-binding domain; CaMBD, CaM-binding domain. *LEA, late embryogenesis abundant At1g64065-like; PPO1, polyphenol oxidase I; RBOH—C, respiratory burst oxidase homolog protein C-like; PIP1–2, aquaporin PIP1–2; SAP, senescence-associated protein; WSC, cell wall integrity/stress response component-like protein; GA3OX1, gibberellin 3-beta-dioxygenase 1-like; ARF17, auxin response factor17;* and *DLO1, protein DMR6-LIKE OXYGENASE 1.*

the highest number of phytohormone response (abscisic acid, ethylene, and auxin) CAREs. In addition, stress-responsive (STRE, ARE, DRE, LTR) CAREs were also predominant in *SiCAMTA2* and *SiCAMTA5* (Figs. 5**A and B**). The expression level of *SiCAMTA2/4a/5* was up-regulated during drought stress in the -tolerant genotype (ZZM0635). This indicates these *CAMTAs* could serve as key regulators for imparting drought tolerance in sesame (Figs. 6**A and B**). However, the down-regulation of *SiCAMTA3* further suggests their involvement in suppressive regulation of drought stress in sesame (Figs. 6**A and B**). Further, the up-regulation of *SiCAMTA2/3/4a/5* and down-regulation of *SiCAMTA4b* in the -tolerant genotype (ZZM2541) under waterlogging stress at 48 h suggest that they might play essential roles in the regulatory network of waterlogging tolerance in sesame (Figs. 6**C and D**).

The enrichment of *CAMTA* recognition motifs in the promoter sequences of stress-responsive genes such as *LEA, PPO1, RBOH—C, DLO, WSC, PIP1–2, SAP, GA3OX1*, and *ARF17* links *CAMTA's* role in the regulation of these genes during drought and waterlogging stresses in sesame (Kim et al., 2013; Pandey et al., 2013). These genes likely play roles in various stress response pathways, such as antioxidative defense (*PPO1*), water transport (*PIP1–2*), reactive oxygen species (ROS) scavenging (*RBOH—C*), cell wall reinforcement (*WSC*), stress signaling (*SAP, ARF17*), osmoprotection (*LEA*), and hormone regulation (*GA3OX1*). Further, specifically, up-regulation of *PPO1, SAP, DLO, LEA, ARF17*, and *GA3OX1* in -tolerant genotype ZZM0635 at the initial stage of drought stress (d1 and d2) suggests they are early-responsive genes to cope with drought stress in sesame (Fig. 9**E and G**). However, downregulation of these genes during prolonged drought stress (d3) could be part of a broader response aimed at mitigating further damage or reallocating resources towards survival mechanisms (Fig. 7**A-B**). Recently, the *RBOH-D* and aquaporin *PIP2;1* genes were suggested to be involved in waterlogging tolerance in *A. thaliana* (Peláez-Vico et al., 2023). The up-regulation of genes like *LEA, PIP1–2, RBOH—C, PPO1*, and *GA3OX1* in the -tolerant genotype ZZM2541 during 12 h and 24 h of waterlogging stress indicates their early responsive role and potential in conferring tolerance to waterlogging (Fig. 9**F and H**). However, the down-regulation of *SAP* at 48 h of waterlogging stress suggests a potential shift in gene expression dynamics as the stress persists. It is also possible that other genes or pathways become more prominent at 48 h of waterlogging stress, contributing to the plant's response to prolonged stress (Fig. 7**C-D**). In addition, SiCAMTAs PPI network also suggested their involvement in regulation of downstream genes related to abiotic (ICS1, ZAT12, DREB1C, and RD29*)* and biotic (SARD1*,* and EDS1*)* stress regulation through the calcium-dependent kinases (CRLKs/CPKs) (Fig. 8). Together, these results show that functional genomic studies on *SiCAMTA* genes reported in the study will help identify appropriate germplasm expressing these genes to impart drought and waterlogging stress tolerance in sesame improvement.

5. Conclusion

In summary, our study identified and comprehensively analyzed the structural and functional role of five *SiCAMTA* genes, with special reference to drought and waterlogging stress, identified in the sesame genome. The expression profiles of *SiCAMTA*s and target genes during drought and waterlogging stress were studied. The outcomes revealed that *SiCAMTA* genes play a pivotal role in orchestrating abiotic stress tolerance in sesame and these results obtained through RNA-seq were validated using qPCR analysis. Our study had highlighted *SiCAMTA2, SiCAMTA5, LEA, PPO1, GA3OX1, RBOH—C*, and *SAP* as potential candidate genes for improving multiple abiotic stress tolerance in sesame. A prospective molecular mechanism of regulation for *SiCAMTA* and its target genes for imparting tolerance to drought and waterlogging stress is summarized in Fig. 10. These findings aim at leveraging these genes to improve stress resilience in sesame.

CRediT authorship contribution statement

Ajay Kumar: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Tamanna Batra:** Formal analysis. **Harinder Vishwakarma:** Investigation, Formal analysis. **Rasna Maurya:** Formal analysis. **Pradeep Ruperao:** Investigation, Formal analysis. **Rashmi Yadav:** Resources, Funding acquisition. **Rajkumar Subramani:** Resources, Funding acquisition. **Gyanendra Pratap Singh:** Resources, Funding acquisition. **Parimalan Rangan:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

Authors hereby declare that there are no competing interests to disclose, both financial and non-financial.

Data availability

The data pertaining to the manuscript is provided as supplemental information.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100532.

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