

Evolutionary and Expression Analysis of CAMTA Gene Family in Three Species (*Arabidopsis*, Maize and Tomato), and Gene Expression in Response to Developmental Stages

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Research Article

Keywords: CAMTA gene family, gene expression, conserved domain, gene structure

Posted Date: July 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-678620/v1>

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Abstract

The calmodulin-binding transcriptional activator (CAMTA) family has been known to be one of the fast responsive stress proteins. In this study, 17 *CAMTA* genes were selected in *Arabidopsis*, tomato and maize. The chromosomal distributions, gene structures, duplication patterns, phylogenetic tree, and developmental stage of the 17 *CAMTA* genes in the three species were analyzed to further investigate their functions. According to the synteny analysis, *CAMTA* genes of maize and tomato revealed higher similarity with each other as compared with *Arabidopsis*. A higher than 90 percent identity was observed between maize *CAMTA* genes (*ZmCAMTA2* and *ZmCAMTA3*) and tomato *CAMTA* genes (*SICAMTA4*, *SICAMTA4.1*). To detect expression levels in different plant tissues, mRNA analysis of *CAMTA* genes were performed using publicly available expression data in the genvestigator. The aim of study was to identify and characterize *CAMTA* genes in three species, for the first time, via insilico genome-wide analysis approach. *AtCAMTA1* and *AtCAMTA2* and *SICAMTA2* and *ZmCAMTA1* and *ZmCAMTA2* genes were up-regulated during all developmental stages. The conserved motifs and gene structure in most proteins in each group were similar, validating the *CAMTA* phylogenetic classification. This study could be considered as a useful source for future *CAMTA* comparative studies in different plant species.

Introduction

Plants are exposed to a variety of stress conditions such as abiotic and biotic stresses during their growth life cycle. These environmental conditions may activate pathways or mechanisms of signaling [1]. To identify responsive genes and transcription factors involved in environmental stress adaptation, interaction of TF with cis-elements was surveyed [2]. Due to the importance of TFs in regulation of stress related genes, increasing number of studies are being performed focusing on their functions. Several studies have shown that TFs affecting life cycles of organisms play a crucial role in plant and cell signaling [3].

In general, over 90 TFs have been known as CaM-binding proteins (CBPs), including CAMTAs, MYBs, WRKY, NACs, and MADS box proteins [4]. One of the most important TFs is calmodulin-binding transcriptional activators (CAMTA), containing four functional domains known as IPT/TIG (transcription factor immunoglobulin), CaMBD, and a varying number of IQ motifs (calmodulin-binding), CG-1 (a DNA-binding domain specific to sequence), and ankyrin (ANK) repeats. Calmodulin-binding transcription activators (CAMTAs), the well-studied CaM-binding transcription factors, are found in animals, fungi, and plants [5]. The binding site for CAMTAs are present in downstream promoter of genes and are designated as A/C/G)CGCG(T/C/G) or (A/C)CGTGT, which helps to regulate their expression [6].

The first CAMTA gene member, NtER1, was identified in tobacco while surveying CaM-binding proteins [7]. In *Arabidopsis*, six CAMTA transporters, termed as AtCAMTA1 to AtCAMTA6, have been detected. These AtCAMTAs are involved in biotic, abiotic, and hormonal regulations and developmental stages [8]. For example, *AtCAMTA1* and *AtCAMTA3* genes play an important role in response to cold and drought stress and in regulation of auxin and salicylic acid in plants. AtCAMTA6 genes are expressed in plants in

response to salt stress, while AtCAMTA4 performs pivotal role in defense responses of plants against *pathogens*, and in response to ethylene, jasmonate acid (JA), and abscisic acid (ABA) [9]. However, comprehensive analyses of CAMTA proteins from a variety of plant species with diverse phylogenetic relationship are still lacking.

Loss-of-function *CAMTA3/AtSR1* mutants revealed chlorosis and autonomous lesions, and elevated resistance to pathogens. Similarly, the mutant of a rice CAMTA member *OsCBT* gene showed significant resistance to pathogens, indicating that OsCBT might also act as a negative regulator on plant defense. CAMTA3 also played important roles in plant defense against insect herbivore, the regulation of glucose metabolism, and ethylene-induced senescence in *Arabidopsis* [10]. Recently, *CAMTA1*, *CAMTA2*, and *CAMTA3* were reported to function together in suppressing SA biosynthesis and were involved in cold/freezing tolerance by CBF transcription induction [11].

CAMTAs from tomato were identified to be differentially expressed genes (DEGs) during fruit development and ripening stages, indicating that calcium signaling is involved in the regulation of fruit development and ripening through calcium/calmodulin/CAMTA interactions [12]. Recently, 15 *CAMTA* genes were identified in soybean, and expression profile revealed that they were responsive to various stresses and hormone signals [13]. Based on a study, *TaCAMTA4* may function as a negative regulator in response to *Puccinia triticina*, since the gene silencing-based knockdown of *TaCAMTA4* resulted in the enhanced resistance to *P. triticina* race 165 [14].

There is little analysis of CAMTA transcription factors for comparison of *Arabidopsis*, tomato and maize. Therefore, this study focuses on the systematic bioinformatics analysis and expression pattern analysis of CAMTA transcriptional factors in three species. In this study, we report the identification and a comprehensive analysis of the *CAMTA* gene family in *A.thaliana*, *Z.mays*, and *S.tuberosome*. Specifically, comprehensive information is provided on the gene structures, chromosomal locations, and phylogenetic relationships, gene duplications, genes expression during developmental stages, and promoter cis-elements identification of 17 *CAMTA* genes in three species.

Material And Methods

The Arabidopsis information resource (TAIR) database was retrieved to download the sequences of six CAMTA family members from *Arabidopsis thaliana*. BLASTP (E-value was $\leq 1e-7$) search was made against the genome of flax in NCBI database using *Arabidopsis* CAMTA proteins as queries. Pfam(<https://pfam.xfam.org/>), SMART (<http://smart.embl-heidelberg.de/>), and conserved domain database were used to collect, screen out, and filter non-redundant CAMTA sequences for conserved CAMTA domains using Gendoc software. The sequences were evaluated against the potential features of the CAMTA transporters such as the presence of IQ (PF00612), ANK (PF12796), TIG (PF01833), and CG-1 (PF03859) domains. The undesired gene sequences were eliminated manually.

ExPASy server (<https://www.expasy.org/>) was employed to predict the theoretical isoelectric point (pI) and the molecular weight (Mw) of each CAMTA protein.

Phylogenetic Analysis And Gene Structure Of Camta Proteins

CAMTA protein sequences were aligned in Arabidopsis, maize, and tomato by using the Clustal W function of MEGA 7.0 and phylogenetic tree was constructed using MEGA 7, applying the Nei-Joubar joining algorithm with 1000 bootstraps replicates. The Gene Structure Display Server v2.0 (GSDS, <http://gsds.cbi.pku.edu.cn/>) was used to obtain information on the exon – intron of CAMTA proteins [15]. Both genome sequences and the coding sequences were utilized for predicting the positional information of the CAMTA genes using Ensemble plant, chromosomal locations. The sizes (bp) and intron numbers of CAMTA genes were identified.

Analysis of Transcription factor binding sites in the CAMTA promoters

1500 bp upstream of the promotor region of CAMTA was retrieved from NCBI database. The sequences obtained were analyzed using PlantPAN database (<http://plantpan.itps.ncku.edu.tw/>) with default limitations to identify the key TFBS with respect to stress response.

Chromosomal Distribution and Conserved motif of the CAMTA Gene

For locating the CAMTA members on *Arabidopsis*, maize and tomato chromosomes, CAMTA genes were placed on each chromosome according to the physical location of the gene. For exon/intron structural analysis, the genomic DNA and CDS sequences corresponding to each predicted *Arabidopsis*, tomato and maize CAMTA factor gene were downloaded from NCBI database. The CAMTA genes were drawn on all chromosomes and presented with MapChart [16]. The conserved motifs of 17 CAMTA protein sequences were analyzed by MEME program (<http://meme.nbcr.net/meme/cgi-bin/meme.cgi>). Conserved motifs were identified by online MEME (<http://meme.sdsc.edu/meme/meme.html>) program using the full length protein sequences of each CAMTA protein sequences, with the following parameters: maximum number of motifs (6 motifs) and motif width set as 6–100 amino acids.

Expression patterns of CAMTA genes in developmental stages

In order to find DEGs under developmental stages, CAMTA gene expression data was extracted by Genevestigator from *A.thaliana*, *Z.mays*, and *S.tuberosum* database using Affymatrix Arabidopsis/maize /tomato Genome Array platform and ‘Perturbations’ tool. DEGs with p-values < 0.05 and log fold-change values ≥ 2 and ≤ -2 were selected for genes. The fold changes in the expression of CAMTA genes under developmental stages were used to generate gene expression heatmap using genevestigator (<https://genevestigator.com/gv/>) with purple/white color schemes as markers where “purple” and “white” colors represent up and down-regulation of the respective genes.

Identification of orthologous and paralogous CAMTA genes between three species

The orthologous genes in all three species were detected from EnsemblPlants (<https://plants.ensembl.org/index.html>). Orthologous genes in CAMTA proteins were selected when the identity exceeded 70% whereas, the paralogous genes were selected when the identity was more than

85% from EnsemblPlants. Orthologous and paralogous CAMTA genes were obtained using Circos program (<http://mkweb.bcgsc.ca/tableviewer/>).

Results And Discussion

In the present study, we identified 17 members of CAMTA gene family in *Arabidopsis*, maize and tomato, and named them according to their position on the chromosome (Table 1). Bioinformatics analyses such as phylogenetic relationships, domains, gene structures, protein motifs, chromosomal locations, and detection of CAMTA homologous and orthologous genes were performed. The expression profiles in different stages were analyzed. All *S/CAMTA*, *ZmCAMTA*, and *AtCAMTA* genes are listed in Table 1 along with their gene nomenclature, genes details, and amino acid length, molecular weight, and point isoelectric. The SICAMTA TFs vary in amino acid length from 916 to 1041 with a predicted isoelectric point (pl) ranging from 5.50 to 8.89 and molecular weight ranging from 103.94 to 117.42 kDa. The AtCAMTA TFs vary in amino acid length from 845 to 1050 with a predicted isoelectric point (pl) ranging from 5.17 to 8.05 and molecular weight ranging from 96.18 to 117.26 kDa. The ZmCAMTA TFs vary in amino acid length from 842 to 1025 with a predicted isoelectric point (pl) ranging from 6.38 to 8.43 and molecular weight ranging from 94.65 to 114.42 kDa. Three genome sequences and the coding sequences were utilized for predicting the positional information of the *CAMTA* genes using Plazza database v12.1.

Table 1
Information for CAMTA transcription factor family members in *Arabidopsis*, maize, and tomato

Ensemble plant	Other gene name	Duplication	Length	MW	Pi:
Solyc01g057270.2.1	SICAMTA3	-	958	107.79	8.89
Solyc01g105230.2.1	SICAMTA2	-	1041	117.42	5.50
Solyc04g056270.2.1	SICAMTA3.1	-	1022	114.15	6.08
Solyc05g015650.2.1	SICAMTA4	-	936	105.30	6.25
Solyc12g035520.1.1	SICAMTA4.1	-	974	108.98	6.68
Solyc12g099340.1.1	SICAMTA6	-	916	103.94	7.18
AT1G67310.1	AtCMTA4	-	1016	113.06	5.17
AT2G22300.1	AtCMTA3	-	1032	116.11	5.25
AT3G16940.1	AtCMTA6	-	845	96.18	8.05
AT4G16150.1	AtCMTA5	-	923	104.85	7.39
AT5G09410.1	AtCMTA1	Block	989	111.99	6.23
AT5G64220.1	AtCMTA2	Block	1050	117.26	6.39
Zm00001d003958_T007	ZmCAMTA1	Block	996	112.21	6.52
Zm00001d021516_T001	ZmCAMTA2	-	913	101.84	8.43
Zm00001d025235_T001	ZmCAMTA5	Block	865	97.52	7.70
Zm00001d028007_T002	ZmCAMTA4	Block	1025	114.42	6.38
Zm00001d042313_T001	ZmCAMTA3	-	842	94.65	6.93

Phylogenetic relationships, conserved motifs and gene structures of the CAMTA factor family genes in *Arabidopsis*, tomato and maize

To determine the phylogenetic relationships among the different members of the CAMTA proteins in *Arabidopsis*, tomato, and maize, a phylogenetic analysis based on alignments of the 17 CAMTA protein sequences was performed. As shown in Fig. 1, the neighbor-joining phylogenetic tree divided 17 CAMTA genes into three clusters. In cluster I, seven CAMTA factors AtCAMTA1, AtCAMTA2, AtCAMTA3, SICAMTA2, SICAMTA3, SICAMTA3.1 and *ZmCAMTA4* gene were grouped. In cluster II, tomato CAMTA factor (SICAMTA6) clustered with AtCAMTA5, AtCAMTA6, ZmCAMTA2 genes. In cluster III, two tomato CAMTA factors (SICAMTA4, SICAMTA4.1) clustered with four other *AtCAMTA4*, *ZmCAMTA1*, *ZmCAMTA3*, and ZmCAMTA5 genes. In contrast, *CAMTA* genes have been divided into seven subfamilies in *Arabidopsis* and rice [17]. It has been reported that the subfamilies I, II and III of the *CAMTA* genes

simultaneously occur in both dicotyledons and monocots, but the subfamily IV genes did not exist in monocots [18]. Another study showed that CAMTA genes were divided into four (I, II, III, and IV) subfamilies in soybean [18]. The genes within each cluster showed similar exon/intron structures and conserved motifs. To reveal the evolutionary relationship between *Arabidopsis*, tomato and maize we comprehensively analyzed the phylogeny between the orthologs and paralog of CAMTAs of three species. In this study, results of our clustering analysis of CAMTA factors in dicots and monocots showed three subfamilies in the phylogenetic tree, similar results as obtained in soybean [18]. Most of the *AtCAMTA*, *SICAMTA* and *ZmCAMTA* genes were classified in the same subfamily. The number of IQ motifs in all CAMTAs varied from one to three. All CAMTAs contain two IQ motifs in C-terminal (Fig. 2a). This study revealed that CAMTAs share the same domain organization as reported previously [18]. Using the MEME tool, a total of eight conserved motifs were detected in the *CAMTA* genes, and the lengths of these conserved motifs varied from 6 to 50 amino acids. Among them, motifs 2, 3, 4, 5, 7 and 8 were widely identified in all CAMTAs (Fig. 2b). In general, the CAMTA factors in the same clade may have similar functions. The composition of conserved motifs in most proteins of each group was similar, which validated the CAMTA transcription factors phylogenetic classification.

Gene Structure

CAMTA genes of group II were disrupted by the highest numbers of introns i.e. 9–12, while group I and group III were disrupted by 1–12 introns. The fixed number of introns and exons is a conserved character of CAMTAs among *Arabidopsis*, maize and tomato [19].

Study of protein structure is important to understand its mode of action. Using the MEME, the conserved motifs of all CAMTAs protein were analyzed. The plant CAMTA-encoded proteins were characterized with the presence of four functional domains, known as CG-1 (a sequence-specific DNA-binding domain), ANK (ankyrin repeats), IPT/TIG (transcription factor immunoglobulin), and IQ motifs (calmodulin-binding) which, are highly conserved among different plant species. All four CG-1, ANK, IPT/TIG, and IQ domains were detected within each of the *StCAMTA*, *ZmCAMTA*, and *AtCAMTA* CAMTA proteins,

Chromosomal Location And Gene Duplication

Analysis of the chromosomal distribution of *AtCAMTA* genes indicated that the 6 *CAMTA* genes of *Arabidopsis* were located on all chromosomes, while the 6 *SICAMTA* genes of tomato were located on chromosomes 1, 3, 4, and 5 but not on chromosomes 2 and 6, 7, 8, 9, 10, 11 (Fig. 3a and 1b). In maize, *ZmCAMTA* genes were located on chromosomes 1,2,3,4, 7, and 10 but not on the chromosomes 5/6/8/9.

It can be concluded from Fig. 4 that there is tandem duplication in *Arabidopsis*, tomato, and maize. Gene duplication can assist plants to adjust to different environments during their development and growth [20].

Prediction of TFBS in the CAMTA genes

To understand the transcriptional, hormonal, and developmental regulation in response to stress, PlantPAN database was evaluated for the prediction of transcription factor binding sites in 1000 bp upstream promotor region of *CAMTA*. The results showed that there are different known stresses -related cis-elements that existed in the promoter regions of the 17 CAMTA genes.

The *CAMTAs* promoters have various cis-regulatory elements, believed to be involved in responses to environmental and hormonal regulations (Fig. 5). In this study, the elements identified in response to various stresses included bZIP, CAMTA, MYB, bHLH, NF-YA NF-YB NF-YC and AP2/ERF.

Analysis of promoters showed that the bZIP and G-box cis-elements were expressed in response to ABA [20]. WRKY cis-elements are regulated in response to auxin hormone. Our results identified AT-hook, YABBY, and *EIL/EIN3* in the promoter regions of CAMTA. Among surveyed CAMTA, *ZmCAMTA* gene had the maximum cis elements in its promoter region. *ZmCMATA2*, *ZmCMATA4* and *ZmCMATA5* genes had the highest number of cis-elements in their promotor regions. The promoter sequences contained approximately 25 types of elements (Fig. 5).

Previous studies have shown that *CAMTAs* were responsive to diverse stresses and stimuli in *Arabidopsis*, tomato, and soybean [11–21]. Enrichment of *cis*-elements involved in stresses/stimuli response in *CAMTA* promoters suggest that they are likely to respond differently to various stresses and stimuli signals, like other *CAMTAs* in different species.

Within the promoter regions of each of the three species, the abiotic responsive cis elements, AP2/ERF, CAMTA, WRKY and MYB were surveyed. Previous study has shown that WRKY cis-elements is often synergistically linked to the occurrence of responsive bZIP TFs [22]. Several AP2/ERFs play key roles in response to the induction of specific stresses and diverse DNA binding preferences and enable these TFs to integrate responses of multiple stimuli in stress condition [23].

Researchers have proposed that AP2/ERF TFs work in tandem with bZIPs and MYBs to bring about synergistic regulation of cold stress tolerance by controlling ABA mediated gene expression in *Arabidopsis* [24–25]. Therefore, it can be suggested that a network of TFs is involved in co-regulating diverse stress-responsive genes, which potentially form the missing molecular link between primary and specialized metabolism genes under stress conditions [26].

Among the cis elements surveyed, dehydrin was the most cis element found in the CAMTA SICAMTA/AtCAMTA2/ *ZmCAMTA2* genes. Due to the presence of dehydrin, plants are found to respond better to drought stresses. The enrichment of the dehydrin in the promoter regions of most *ZmCAMTA/AtCAMTA/SICAMTA* family genes suggested comprehensive transcriptional regulation by the CAMTAs themselves, and indicated a complicated regulation network between them. Our finding showed that the surveyed *S/CAMTAs* genes, such as SICAMTA7, SICAMTA2, SICAMTA17, and *S/CAMTA11*, were up-regulated under drought stress. Physiologically, the WRKY TFs binding to *W-boxes* regulate various

developmental activities (controlling senescence) and defense associated processes (regulating responses to pathogen infestation and other abiotic stresses) [27]. Similarly, the MYB-binding sites are present in the promoters of the genes (*StCAMTA26a/26b* and *SICAMTA19/5/18*) and MYB (*StCAMTA26a/26b* and *SICAMTA4/ 5 /8/8A/16a/19/26a/26b*). It has been recognized that MYB TFs binding to their respective *cis*-elements control changes in various processes like hormonal signaling, specialized metabolism (phenylpropanoid and anthocyanin biosynthesis), cellular morphogenesis, and formation of meristem [28–29].

Next, we further analyzed the hormone responsive elements in *Arabidopsis*, maize, and tomato *CAMTA* promoters. The most dominant hormone responsive element in *CAMTAs* is ABRE for recognizing ABA signal, detected in the promoters of 17 *CAMTAs*. Another founding member is LOB (corresponding to ASL3), which is intriguing in that it is expressed specifically at the base region of all lateral organs formed from shoot apical meristem (SAM) and floral meristem [2].

The maximum number of WRKY elements was related to AtCAMTA3/6, SICAMTA4 and ZmCAMTA5/6. Therefore, we can conclude that WRKY, in the promoter regions of genes, has important roles in response to pathogens. The ethylene-insensitive3-like/ethylene-insensitive3 (*EIL/EIN3*) protein family can serve as a vital factor for plant growth and development under different environmental conditions. *EIL/EIN3* protein is a form of a contained nuclear protein with DNA-binding activity, contributing to the complex network of primary and secondary metabolic pathways of plants [30]. AT-hook DNA binding proteins are known to contribute to a functional nuclear architecture by binding to the nuclear matrix. AT-hook motifs bind to the minor grooves in duplex DNA of matrix attachment regions (MAR) of target DNA sequences, containing characteristic AT-rich DNA sequences. Many plant AT-hook motif proteins have a plant and prokaryote conserved (PPC) domain with unknown functions. At-hook motifs are related with functional domains in chromatin proteins and in DNA-binding proteins like that homodomains and zinc fingers.

Our finding showed that YABBY, WRKY, and GATA are involved in response to developmental stages and abiotic stresses. The NAC and YABBY transcription factors are known to be involved in numerous biological processes [32]. Another group of transcription factors is YABBY, playing a critical role in determining organ polarity. It is involved in the establishment of abaxial-adaxial polarity in lateral organs [32]. YABBY family transcription factors contain a zinc-finger domain in the amino-terminal region and a YABBY domain in the carboxyl-terminal region.

Orthologous And Paralogous Genes Study In Camta Transcription Factor

In the present study, a comparative analysis was performed to identify orthologs of *CAMTA* genes between *Arabidopsis*, maize and tomato genomes (Fig. 6). Based on the results, AtCAMTA5 with AtCAMTA6; AtCAMTA2 with SICAMTA3.1 and SICAMTA4 with SICAMTA4.1 genes showed high similarity (identity 70%) and in total resulted in three orthologous gene pairs. The analysis of synteny showed that

ZmCAMTA1 with ZmCAMTA3 were paralogous. The syntenic analysis suggested the segmental and tandem duplication as a major force for the diversity in *CAMTA* gene family, revealing the structural and functional conservation of the genes underlying the origins of evolutionary novelty. Further, orthologs with similar functions have conserved domains [31].

No duplicated *CAMTA* genes were identified from synteny analysis in maize, implying different expansion types of this gene family between maize and dicots *Arabidopsis* and tomato, but *AtCAMTA* with *S/CAMTA3.1* showed high similarity. Based on our findings, a comparative analysis was performed to identify orthologs of *CAMTA* genes among three genomes. Orthologous genes among *Arabidopsis*, maize, and tomato suggested that whole genome duplication (polyploidy) plays an important role in the expansion of *CAMTA* genes. In the pathway, gene duplication consisted of chromosomal duplications.

In addition, chromosomal/segmental duplication in *Arabidopsis*, maize and tomato genomes, was detected. Often orthologous genes have a similar function among different species. Therefore, the study of evolutionary genomics can shed light on the gene function. Analysis of *CAMTA* genes showed that whole genome duplication, tandem duplication, and chromosomal/segmental duplications play an important role in tomato genome expansion. However, the number of tandem/segmental duplications indicate that they are main factors in the evolution of *CAMTA* genes. Given the main role of these three species as model plants, their genomes provide a new resource for use in breeding. Also, three orthologous gene pairs were identified between *A.thaliana* and *Z.mays*.

Analysis of Expression Pattern of CAMTA Transcription Factors during developmental stages

Analysis of expression pattern of CAMTA in *Arabidopsis*, tomato and maize under developmental stage were performed. As shown in Fig. 7a 7b and 7c in *Arabidopsis*, tomato and maize, respectively, we can clearly see the expression of 6 *AtCAMTA* transcription factors in developmental stages of *Arabidopsis* that AtCAMTA5 and AtCAMTA6 were up-regulated in almost of all developmental stages such as senescence and young rosette (Fig. 7a). In tomato, As shown in Fig. 7b, most of *S/CAMTA3* genes down-regulated gene, which up-regulated SICAMTA2, SICAMTA3, SICAMTA3.1, SICAMTA4, SICAMTA4.1, and SICAMTA6 in developmental stages. CAMTA TFs are regulated at many processes of plant growth and development, especially during light-mediated processes such as flowering, maturation, embryo development, and differentiation and expansion [33]. In maize, *ZmCAMTA1* and *ZmCAMTA2* genes were up-regulated in all developmental stages. It has been reported that some *ZmCAMTA* genes were expressed in different tissues. The CAMTA genes highly expressed in organs of plants are crucial for the functioning or development of a specific organ. *StCAMTA11* and *StCAMTA28* genes up-regulated in root, shoot, and inflorescence. It has been reported that fewer differentially expressed CAMTA factor genes were found in soybean roots than in soybean leaves [35]. CAMTAs have been shown to be extensively involved in plant growth and developmental regulation, as well as in biotic and abiotic stress tolerance [34]. In *Arabidopsis*, *CAMTA1* and *CAMTA2* genes use in concert with *CAMTA3* to directly bind to the promoter of C-repeat binding factor2 (*CBF2*) to induce expression, leading to increased plant freezing tolerance. AtCAMTA3 can act as a negative regulator of plant immunity to modulate pathogen defense

responses by activating the EDS1-mediated salicylic acid (SA) signaling. A recent study showed that *TaCAMTA4* may function as a negative regulator of the defense response against *Puccinia triticina*, since the virus-induced gene silencing (VIGS)-based knockdown of *TaCAMTA4* resulted in the enhanced resistance to *P. triticina* [18]. Our results suggest that one CAMTA member usually participates in multiple signaling pathways, while multiple CAMTA members often work together to participate in one signaling pathway.

Conclusions

In conclusion, 17 *CAMTA* genes were identified in *Arabidopsis*, tomato, maize in the present study. Analysis of the gene structure and protein domain, biochemical properties, and the phylogenetic tree indicated that the *CAMTA* gene family was highly conserved during plant evolution. Expression analysis showed that all 17 *CAMTA* genes were expressed in multiple tissues with different expression levels, suggesting that various *CAMTA* gene members maintain different functions in growth and development. Using prediction of cis elements, the presence of AP2/ERF in all the *CAMTA* genes could respond to at least one abiotic stress or multiple stresses, implying different regulations and functions of *CAMTA* gene members for coping with various abiotic stresses. *CAMTA* genes in *Arabidopsis*, tomato, maize genome was predicted to be potential target genes by *CAMTA*, demonstrating that *CAMTA* can be widely involved in plant development and growth, as well as coping with stresses. Our findings provide new insight into the *CAMTA* gene family in three species as well as a foundation for further studies on the roles of *CAMTA* genes in wheat development, growth and stress response. This study will help to identify novel *CAMTA* genes for future breeding programs to improve plant production, quality and stress resistance, and open a new way for further elucidation for their roles underlying the signal transduction among these plant species.

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Figures

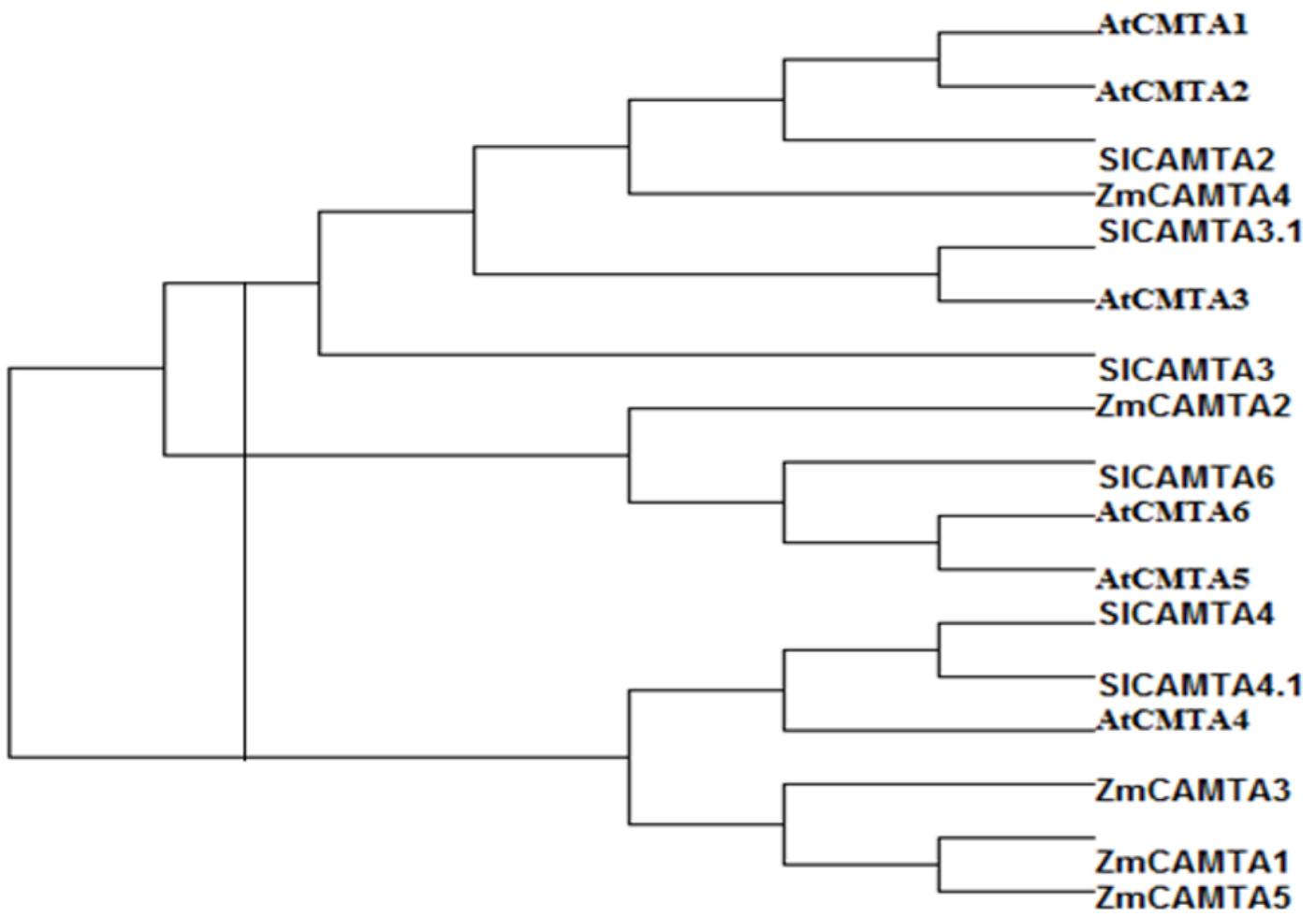
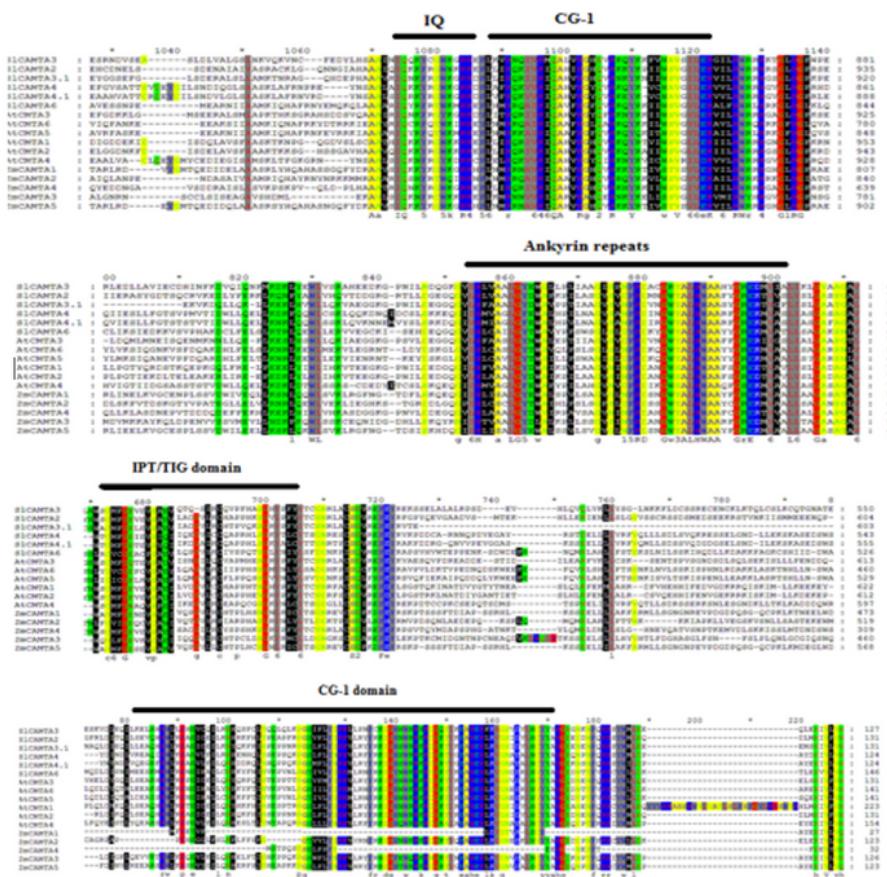
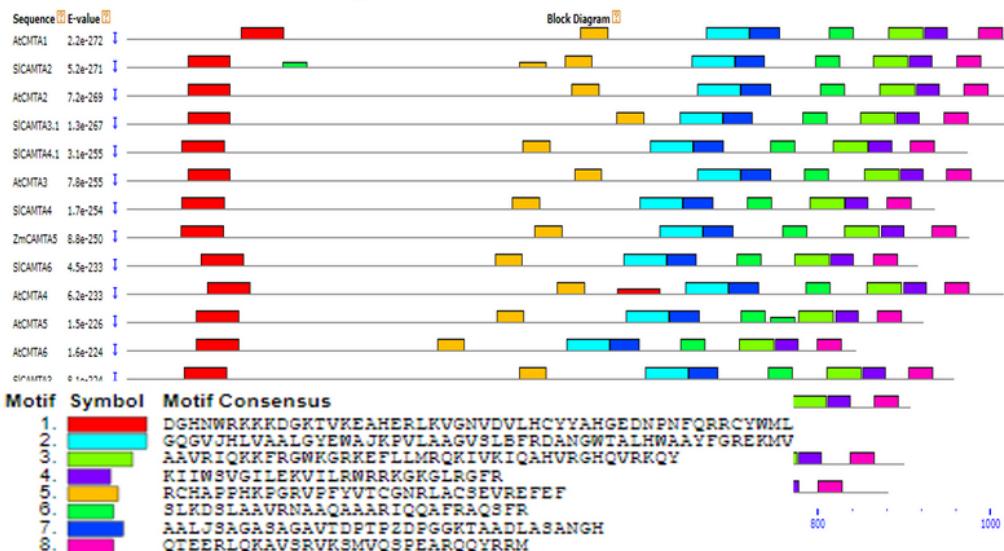


Figure 1

Phylogenetic tree of CAMTA genes created by the neighbor-joining (NJ) method in MEGA7.0 software.



a



b

Figure 2

a. Multiple alignment of Arabidopsis, maize, and tomato CAMTA family proteins. Multiple alignment was performed via Clustal W and the residues were colored using Genedoc software. b. Conserved CAMTA protein motifs in maize, recognized by MEME database. Motifs 1-8 are identified by different colors.

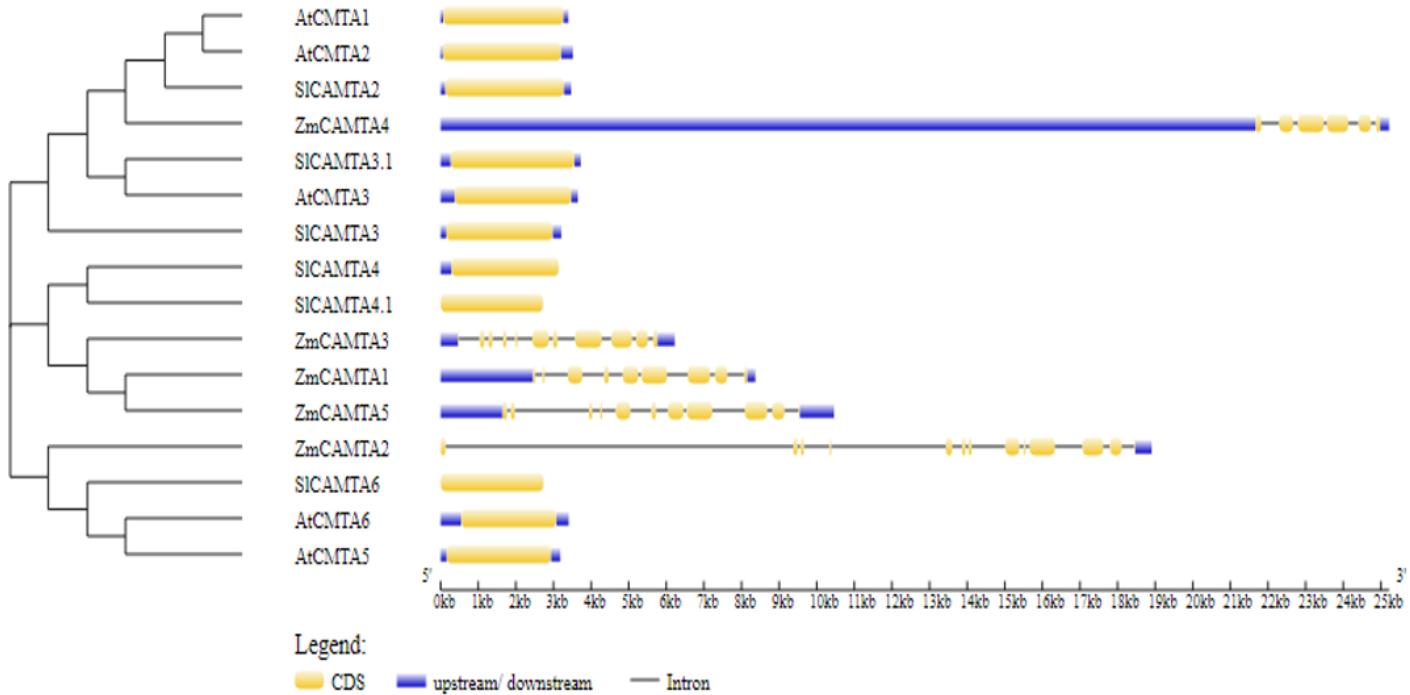


Figure 3

The exon-intronic structure of *A. thaliana*, *Z. mays*, and *S. tuberosum* CAMTA genes according to their phylogenetic relationships. Yellow and blue colors represent gene exon and intron, respectively.

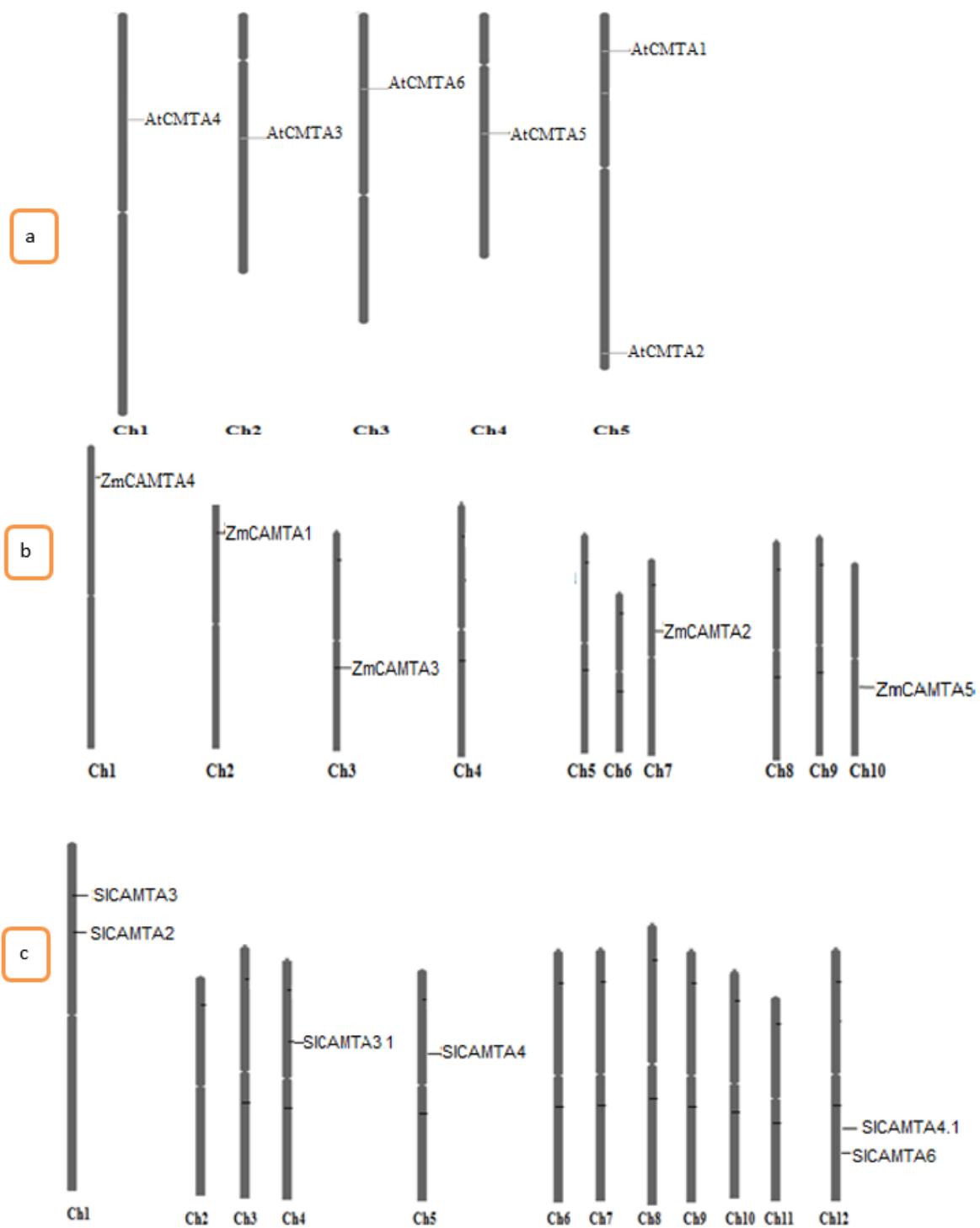


Figure 4

Chromosomal distribution and expansion patterns of CAMTA genes in Arabidopsis (a), maize (b), and tomato (c).

	MYB;ARR-B	Myb/S	C2H2	Homeodomain	bZIP	HD-ZIP	MYB-Dof	GATA	MYB	EIN3;E	TCR	AT-H	bHLH	Trihelix	NAC	SBP	NF-YB;NF-Y	Dehydr	TCP	Sox;YABBY	WRKY	LOB	MADS box	AP2;ERF			
AtCAMTA4	28	100	24		62	136	39	12	118	190	80	7	4	8	98	89	12	44		101	45	8	2	59	2	9	126
AtCAMTA3	25	91	17		86	168	46	22	135	197	95	4	11	24	125	110	11	73		129	42	8	0	101	0	4	151
AtCAMTA6	58	158	41		74	120	42	34	119	277	120	10	0	30	74	90	13	46		121	41	5	0	102	2	7	161
AtCAMTA5	44	105	27		41	144	13	18	88	208	94	1	1	24	99	88	22	43		90	57	6	0	69	1	16	120
AtCAMTA1	37	91	30		56	172	29	29	126	207	100	9	9	26	146	106	25	29		108	51	17	2	64	2	4	135
AtCAMTA2	32	80	33		81	178	39	20	102	218	105	7	13	21	103	86	12	55		133	62	15	2	77	1	10	169
SiCAMTA3	108	33	73		178	51	15	110	179	100	4	18	22	74	90	19	49	114		33	2	2	90	1	1	144	100
SiCAMTA2	41	120	23		93	149	52	18	105	213	134	7	17	23	87	91	19	21		147	38	8	2	48	2	5	156
SiCAMTA3.1	33	116	34		90	141	56	8	112	187	90	5	10	21	105	99	20	45		156	51	19	2	90	0	6	155
SiCAMTA4	40	121	30		106	235	74	18	82	219	143	1	10	14	242	71	9	62		117	54	14	0	36	1	5	113
SiCAMTA4.1	34	93	17		85	196	54	25	70	178	125	3	13	16	162	68	18	43		95	44	3	0	74	0	4	109
SiCAMTA6	32	98	36		61	61	28	18	62	204	100	1	3	24	103	61	16	37		100	40	15	0	60	3	12	143
ZmCAMTA1	70	208	70		160	121	73	29	235	406	264	27	17	118	31	6	26	115		50	316	42	3	151	3	16	356
ZmCAMTA2	356	359	135		329	1096	192	72	447	899	495	48	33	180	716	465	77	330		621	464	147	6	287	4	39	1008
ZmCAMTA5	98	308	106		222	455	112	55	313	588	388	29	42	175	246	315	46	151		382	136	52	4	293	2	26	584
ZmCAMTA4	192	598	164		445	1100	239	95	571	1079	732	37	40	204	955	621	124	423		872	704	215	14	330	12	53	1460
ZmCAMTA3	49	123	45		165	368	116	22	172	352	130	23	18	61	456	185	30	116		234	83	17	4	65	5	13	322

Figure 5

Prediction of TFBS in the promoter regions of CAMTA genes in Arabdopsis, tomato, and maize.

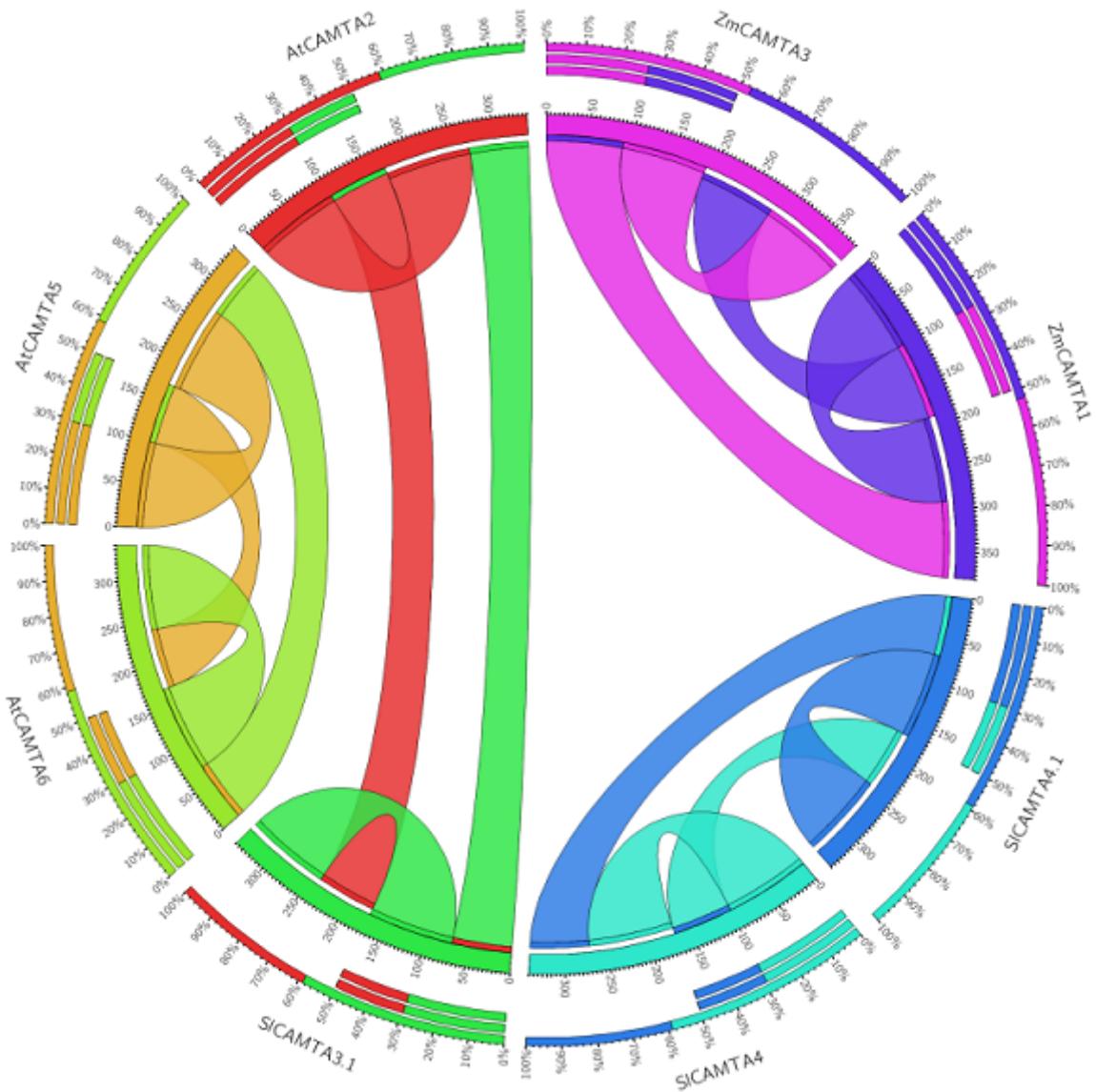


Figure 6

Orthologous and Paralogous relationships of CAMTA genes with Arabidopsis, maize, and tomato genomes visualized by Circos database.

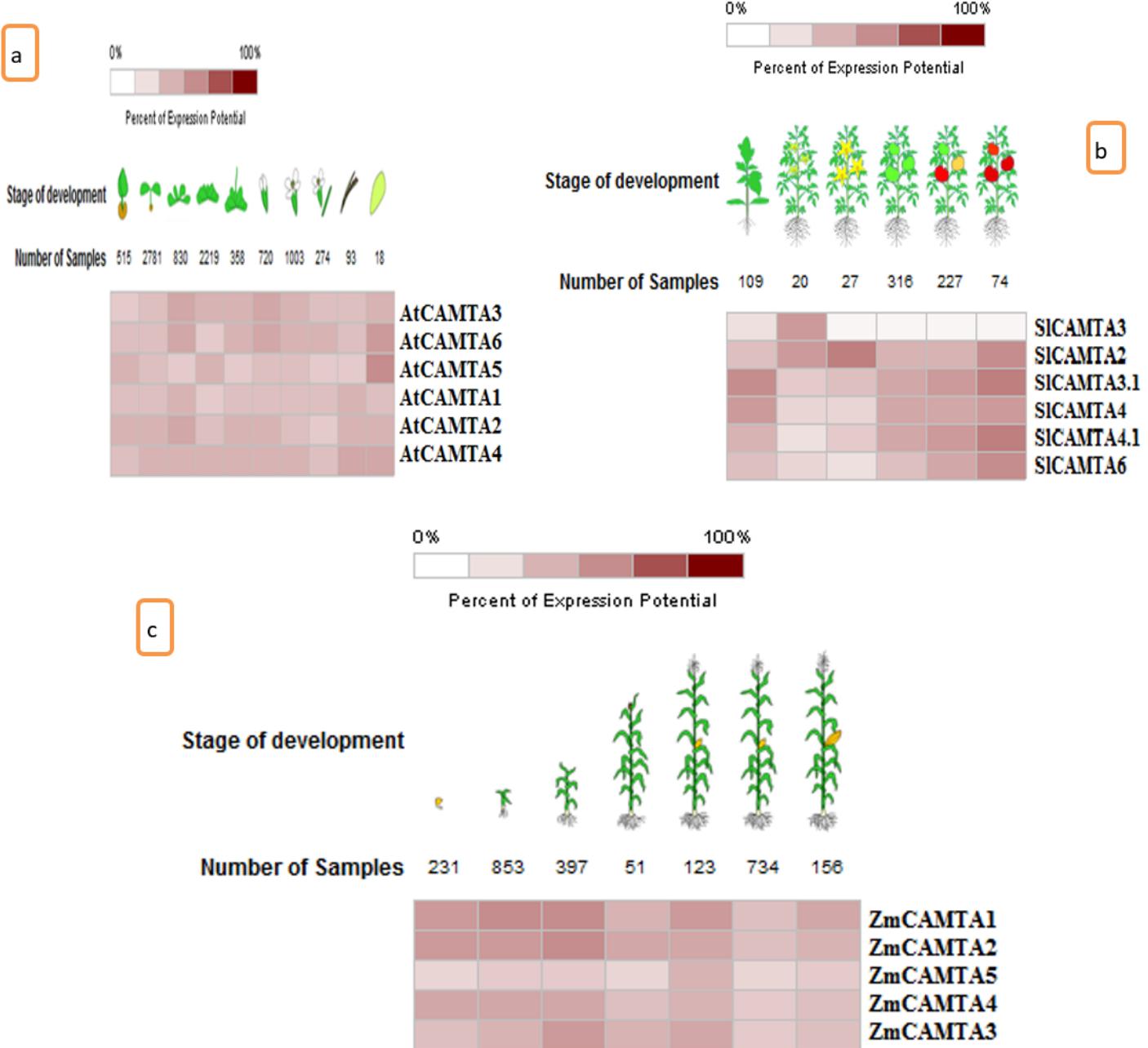


Figure 7

Heatmap representation of expression analysis of CAMTA genes at different developmental stages of *Arabidopsis* (a), tomato (b), maize (c).