Comprehensive identification and expression analysis of CAMTA gene family in *Phyllostachys edulis* under abiotic stress (#79685)

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Comprehensive identification and expression analysis of CAMTA gene family in *Phyllostachys edulis* under abiotic stress

Ce Liu [1, 2], **Dingqin Tang** [Corresp. 1, 2]

[1] School of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou City, lin'an, China

[2] State Key Laboratory of Subtropical Forest Cultivation, Zhejiang A&F University, Hangzhou City, Lin'an, China

Corresponding Author: Dingqin Tang
Email address: tang@zafu.edu.cn

Background. The CAMTA family are major transcription factor regulated by calmodulin (CaM) that play an essential role in plant growth, development and response to biotic and abiotic stresses. The CAMTA gene family has been identified in Arabidopsis thaliana, rice and other model plants, and its gene function in moso bamboo (*Phyllostachys edulis*) has not been identified.

Results. In this study, a total of 11 CAMTA genes were identified in the moso bamboo genome. Conserved domain and multiplex sequence alignment analysis showed that the structure between these genes was highly similar, with all members having CG-1 domains and some members having TIG and IQ domains. Phylogenetic relationship analysis showed that the CAMTA gene was divided into 5 subfamilies, and gene fragment replication promoted the evolution of this gene family. Promoter analysis revealed a large number of cis-acting elements associated with drought stress in PHCAMTA, suggesting that this family is involved in drought stress. Abiotic stress on moso bamboo was also found to be involved in drought stress response, which was similar to the results of promoter analysis. Gene expression pattern according to transcriptome data revealed participation of the PHCAMTA genes in tissue development.

Conclusions. Our results present new findings for the moso bamboo CAMTA family and provide partial experimental evidence for further validation of the function of PHCAMTAs.

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State Key Laboratory of Subtropical Forest Cultivation, Zhejiang A&F University, Hangzhou, Lin'an, China
School of Forestry and Biotechnology, Zhejiang A&F University, Zhejiang, Lin'an, China

Corresponding Author:
Dingqin Tang
666 Wusu Street, Lin'an District, Hangzhou City, Zhejiang Province, 311300, China
Email address: tang@zafu.edu.cn

Abstract

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Introduction

Calcium (Ca^{2+}) ions are involved in many cellular signaling pathways as prevalent secondary messengers in eukaryotes (Wu et al. 2016). Ca^{2+}-mediated signaling plays a key role in the transmission of signals generated by different stimuli, thus mediating various stress responses in plants (Evans et al. 2001; White & Broadley 2003). CaM is a ubiquitous eukaryotic Ca^{2+} sensor that binds Ca^{2+} into a flexible Ca^{2+}/CaM structural protein, which, together with the ability of Ca^{2+} to interact with a number of proteins, allows CaM to regulate protein targets in many different signaling pathways (Bouché et al. 2005; DeFalco et al. 2016; Poovaiah et al. 2013; Yamniuk & Vogel 2004). Ca^{2+} and CaM complexes deliver various endogenous and exogenous signals through multiple interactions with transcription factors (TFs) in response to plant responses (Kim et al. 2009). CAMTA, a major transcription factor regulated by calmodulin (CaM), was first identified in tobacco in 2009 (Kim et al. 2009). The CAMTA protein structural domain contains the following functional domains: (1) N-terminal containing a CG-1 DNA binding domain; (2) A TIG structural domain engaged in non-specific DNA binding; (3) Ankyrin repeat sequences responsible for mediating interactions between different proteins; (4) a Ca^{2+}-dependent CaM binding domain between the N-terminal and C-terminal; (5) IQ motifs interacting with CaM (IQXXXRGXXXR) (Bähler & Rhoads 2002; Bouché et al. 2002; Du et al. 2009; Finkler et al. 2007; Yang & Poovaiah 2002). CAMATA was discovered when part of the cDNA clone (CG-1) was isolated from parsley and subsequently reported in various multicellular organisms (Iqbal et al. 2020).

It has been found that CAMTA transcription factors exhibit very important and simple and effective functions in plant growth and development, biotic and abiotic stress (e. g. low temperature stress) responses, and that CAMTAs of different species respond to various biotic and abiotic stresses including low temperature, hormones, high salt and drought to varying degrees (Chung et al. 2020; Noman et al. 2021; Shkolnik et al. 2019; Yue et al. 2015). The important role of CAMTA3 gene for Brassica napus (cabbage, kale and kale type oilseed rape) in cold and disease resistance was found (Luo et al. 2021). Two genes, ZmCAMTA4 and ZmCAMTA6, were highly expressed in maize under abiotic stress treatment, and cis-element analysis revealed the involvement of CAMTA genes in the association between environmental stress and stress-related hormones (Liu et al. 2021). Ming wei (Wei et al. 2017) suggested that PtCAMTA genes play an essential role in resistance to cold stress, and he showed that woody plants and crops have different CAMTA gene expression patterns under abiotic stresses and phytohormone treatments. The land cotton (Gossypium hirsutum) GhCAMTA11 gene is specifically expressed in roots and under heat stress, and GhCAMTA7 and GhCAMTA14 are also expressed under drought stress, indicating that the land cotton CAMTA gene family is involved in the growth and development process and stress reaction of land cotton (Zhang et al. 2022). It was found that the biochemical response of HbCAMTA3 in response to low temperature stress in rubber trees is similar to that of AtCAMTA3 in Arabidopsis, and that the AtCAMTA3 gene is also involved in salt stress reaction implying that HbCAMTA3 in rubber trees is functionally diverse (Lin et al. 2021). Interestingly, it was found that TaCAMTA mainly responds to drought stress in wheat in reaction to various abiotic stresses in the nursery stage,

and TaCAMTA1b-B. 1 plays an essential role in the response to drought stress caused by water deficit in the nursery stage (Wang et al. 2022).

Phyllostachys edulis is a genus of *Phyllostachys Sieb* in the family Gramineae, which is widely distributed in China and is an important bamboo resource with the characteristics of strong adaptability, rapid growth, easy reproduction and good timber (Lin et al. 2002; Xu et al. 2022; Yang & Li 2017).

We comprehensively analyzed the phylogenetic relationships between moso bamboo and model plants in the CAMTA gene family to elucidate their evolutionary relationships. Using available RNA-seq data and qRT-PCR results, we analyzed the expression profile of PHCAMTA family genes during plant growth and development, as well as the expression of this gene family during stressful abiotic stresses. In this study, we identified the CAMTA gene family in P. edulis in order to provide relevant data support in future plant breeding studies and to open new avenues for further elucidation of its role in P. edulis signal transduction.

Materials & Methods

dentification of CAMTA Genes in *P.edulis*

All files associated with the whole genome sequence data of *Phyllostachys edulis* were downloaded from the database website (http://gigadb.org/dataset/100498). A numerical tabular Hidden Markov Model (Profile HMM) was constructed using HMMER3 (https://myhits.sib.swiss/cgi-bin) to match the *Phyllostachys edulis* protein database (significant E value set to no more than 1×10-20) (Finn et al. 2011). The CAMTA domain (PF03859) obtained from the Pfam database was screened and integrated (Finn et al. 2016), and the candidate gene family members were obtained from the initial screening. The CAMTA structural domains of the candidate family members were analyzed using SMART (Letunic et al. 2012), along with the Plant TFDB and NCBI BLAST for further comprehensive analysis and identification to obtain candidate CAMTA transcription factor families (Jin et al. 2017).

Physicochemical properties and signal peptide analysis of *P.edulis* CAMTA

The Sequence Toolkits module of TBtools software (v1.098765) was used to derive the coding sequence (CDS), protein fasta sequence, and gene structure and location information of CAMTA family members from the corresponding genome-wide database (Chen et al. 2020) using The online tools Prot Param and TargetP 2.0 Server (https://services.healthtech.dtu.dk/service.php?TargetP-2.0) were used to analyze their physicochemical Properties, signal peptides were analyzed.

Interspecific evolutionary analysis of gene families

The whole genome information of rice, Arabidopsis, Zea, and Brachypodium distachyon. was downloaded from the rice genome database, the Arabidopsis database(http://www.arabidopsis.org), Zea database(http://www.arabidopsis.org), and Brachypodium distachyon. database(http://plants.ensembl.org/), respectively, and based on the obtained CAMTA Protein sequences of the 4 plants, the software ClustalX2.1 was used to contrast the CAMTA Protein sequences of M. spp. The sequence alignment results were used to

construct phylogenetic trees by the software MEGA7 using the neighbor-joining (NJ) method, and the bootstrap evaluation (Bootstrap) was repeated 1000 times.

Gene structure, conserved structural domains and motif analysis

Based on the gene location information of *P.edulis* genome annotation file (GFF), the gene intron and exon sequences were analyzed and the gene structure of PHCAMTA family was visualized; the NCBI online software CDD was used to forecast the conserved structural domains of CAMTA family members, and their amino acid conserved sequences were predicted using the online software MEME (Bailey et al. 2009).

Cis-acting elements in the PHCAMTA gene promoter regions

Extract the first 1500 bp sequence of the promoter of PHCAMTA family gene, predict it online using Plant Care, and submit the results to TBtools (v1.098765) Visualization.

ChroMosome distribution and interspecies covariance analysis

The BLAST module of TBtools (v1.098765) software was used to execution sequence comparison of all proteins in the genome of bamboo, and two-way alignment of Moso bamboo with rice and Moso bamboo with Arabidopsis, based on genome-wide GFF files, using MC ScanX, Circos (0.69-9) and Multipe Synteny Plot. CAMTA family chroMosome distribution and interspecies covariance were visualized using MC ScanX, Circos (0.69-9) and Multipe Synteny Plot.

Tissue-Specific Expression Levels of PHCAMTA Genes

In order to analyze the specific expression of CAMTA gene in P. chinensis chinensis, we downloaded RNA-seq data from the NCBI gene expression profiles database (Accession: ERR105067-ERR105076). Transcriptome data, quantified as transcripts per million reads (TPM), and log2-transformed (Cushion et al. 2018).

Plant Material, RNA extraction and qRT-PCR analysis

Normal-grown 3-month-old live Moso bamboo seedlings were used as the control group with the following abiotic stress treatments: 4 °C and 500 ml 30% PEG6000; sampled at 0, 3, 6, 12 and 24 h for the above treatments, and at 0, 3, 6 and 12 h for 42 °C-treated live Moso bamboo seedlings, and the second youngest leaf from top to bottom was snap-frozen in liquid nitrogen and saved in a -80 °C freezer.

Extraction of total RNA using an RNA extraction kit (Kangwei Century Biotechnology Co., Ltd.). cDNA was synthesized using Ta Ka Ra's SriciptTM RT kit and used for subsequent qRT-PCR assays. For the 11 identified PHCAMTA genes, qRT-PCR primers were designed online using Primer Premier 3, with Moso bamboo NTB (nucleotide tract-binding protein) as the internal reference gene (Fan et al. 2013). SYBR qPCR Master Mix (Code. Q311-02, Nanjing, China) was used to perform qRT-PCR in Multiplate™ 96-well PCR plates (Bio-Rad, California, USA). Each sample was tested using three technical replicates to ensure the accuracy of results. The reaction conditions refer to the method of Ma R (Ma et al. 2021).

Results

Identification and characterization of PHCAMTA genes in *P.edulis*

Eleven candidate family members were searched by the plant CAMTA Pfam (PF04770) model, and a significant E value of no more than 1×10-20 was set for preliminary screening. 11 CAMTA family members were obtained by combining gene structure, chroMosomal localization, conserved structural domains and other characteristics, and removing gene duplicate transcripts and non-full-length amino acid sequences. As shown in Table 1, the CAMTA family genes were renamed PHCAMTA01 to PHCAMTA11 based on the chroMosomal positioning information of the genes. bioinformatics analysis of the protein sequences of the 11 family members showed that the largest protein molecular weight of the CAMTA family members was 114.92 kD, and the smallest protein molecular weight was 90.10 kD. The amino acid sequence lengths ranged from 816 to 1031aa. The isoelectric points lie between 5.18 and 8.2. Two of the family proteins are basic (theoretical isoelectric point >7) and nine are acidic (theoretical isoelectric point <7). The aliphatic amino acid index revealed that the thermal stability of the proteins of this family was between 74.03 and 80.82, suggesting that the proteins of this family have small differences in thermal stability. Signal peptide analysis showed that none of the 23 members had signal peptides, indicating that the protin sequences of the CAMTA genes of *P.edulis* do not have transmembrane structures.

Phylogenetic analysis

In reference to (Dezhou Wang) (Wang et al. 2022), the amino acid sequences of Mao bamboo CAMTA, Rice CAMTA, Arabidopsis CAMTA, Zea CAMTA and *B.distachyon* CAMTA were subjected to phylogenetic analysis. The analysis revealed that the amino acid sequences of PHCAMTA could be classified into five subclades (Ⅰ~Ⅴ) (Fig 1), among which the protein sequences of Arabidopsis CAMTA genes were classified into one subclade, and the amino acid sequences of rice, Zea, *P.edulis* and PHCAMTA genes were grouped into one subclade. It is more closely related to rice and Zea, and more distantly related to Arabidopsis.

Gene structure, conserved domains, motifs and sequence analysis

Analysis of the gene structure of PHCAMTA family showed that the number of introns (intron) of each PHCAMTA gene ranged from 10 to 14. The 11 sequences were divided into four categories, because the affinities of *P.edulis* in other species make the results differ from the classification in the evolutionary tree. Gene PHCAMTA09 in subfamily III contains the longest intron region, while gene PHCAMTA04 in subfamily II and gene PHCAMTA11 in subfamily III have the shortest introns.

PHCAMTA gene family was further analyzed for conserved structural domains based on the NCBI online software CDD, as shown in Fig 2C. As shown, all CAMTA family members contained CG-1 structural domains located at the N terminus, PHCAMTA10, PHCAMTA05, PHCAMTA03 in the first subclade and PHCAMTA11, PHCAMTA06 in the third subclade had TIG structural domains in addition to the typical CG-1 structural domains, while PHCAMTA08 in the first subgroup and PHCAMTA09 in the third subgroup do not have TIG structural domains, and both the second and fourth subgroups contain both CG-1 and TIG structural domains. All of the first subgroup contained ANKYR structural domains, PHCAMTA07 in the second subgroup and PHCAMTA09 and PHCAMTA06 in the third subgroup contained ANKYR

198 structural domains, and the rest of PHCAMTA01 and PHCAMTA04 in the second subgroup,
199 PHCAMTA11 in the third subgroup and the fourth subgroup did not contain ANKYR structural
200 domains. All of the second subclade contained IQ structural domains, PHCAMTA08,
201 PHCAMTA10, PHCAMTA03 in the first subclade, PHCAMTA11, PHCAMTA09 in the third
202 subclade and the fourth subclade contained IQ structural domains, and the remaining
203 PHCAMTA05 in the first subclade and PHCAMTA06 in the third subclade did not have IQ
204 structural domains. PHCAMTA08, PHCAMTA10, PHCAMTA03 in the first subfamily and
205 PHCAMTA07 in the second subfamily have all CAMTA structural domains.
206 The members of the Mauve CAMTA gene family contain motifs numbering 6 and 8, which are
207 highly conserved, of which motif1, motif6 and motif7 constitute the CG-1 structural domain.
208 Except for PHCAMTA05, which lacks motif7 and motif3 in the first family, the genes in the
209 other families have all motifs.
210 **ChroMosomal location and gene duplication of PHCAMTA genes**
211 The chroMosome distribution of the PHCAMTA gene family showed that 11 CAMTA genes
212 were distributed on nine chroMosomes with different chroMosome gene division densities, and
213 only genes PHCAMTA08 and PHCAMTA10 underwent gene doubling (tandem duplication),
214 while the rest of the genes did not show gene duplication. The results indicated that only
215 individual genes caused amplification of CAMTA transcription factor members on different
216 chroMosomes through gene duplication.
217 As shown in Fig 4, no CAMTA homologous protein genes of Moso bamboo occur in pepper
218 chroMosomes, only three Moso bamboo CAMTA homologous protein genes occur in
219 Arabidopsis chroMosomes, while 14 Moso bamboo CAMTA genes can be found on six Zea
220 chroMosomes with corresponding paralogous homologs, and 17 Moso bamboo CAMTA genes
221 can be found on five rice chroMosomes with corresponding paralogous the same genes were
222 found on five rice chroMosomes. Therefore, the covariance between Moso and rice and Zea was
223 more significant than that between Moso and pepper and Arabidopsis. In addition, most of the
224 genes in the rice and Zea CAMTA families have more than two paralogous homologs in Moso
225 bamboo, inferring that there may have been a massive gene doubling event in the Moso bamboo
226 CAMTA gene family in the evolution process.
227 **Cis-element analysis of PHCAMTAs**
228 The Moso bamboo CAMTA family members contain 11 genes extracted upstream to 1500 bp
229 nucleotide sequences, and promoter prediction revealed that in addition to the core promoter
230 elements, many other cis-acting elements were found (Fig 5), such as light-responsive elements,
231 hormone-response-related elements and stress-responsive elements related to plant growth and
232 development. The most abundant were hormone response-related elements, with all gene
233 promoters containing at least one light response element and most gene promoters containing at
234 least one phytohormone response element. The stress response elements include low temperature
235 stress response components, drought stress response components, anaerobic induction response
236 components and other abiotic stress response components. All PHCAMTAs contained the
237 drought stress response component MYC, and the drought stress response component MYB was

the most abundant response element, suggesting that PHCAMTA plays an essential role in drought stress response. The results suggest that different components of the promoter region of Moso bamboo CAMTA gene family may be important in regulating plant growth and development and in resisting abiotic stresses.

Tissue-Specific Expression Levels of PHCAMTA Genes

To study the physiological role of CAMTAs, we analyzed the gene expression patterns of CAMTAs. The expression levels of CAMTAs in four tissues (leaf, stem, whip and root) were assessed by RNA-seq data. Gene expression profiles in different tissues indicated that CAMTA has different functions in moso bamboo. The results showed that PHCAMTA07/11 expression profile was higher than other genes. The expression of Moso bamboo CAMTA was higher in leaves than in stems, whips and roots, except for PHCAMTA11. Moreover, PHCAMTA11 was more highly expressed in each tissue, indicating that this gene plays an important role in the overall development of Moso bamboo.

Expression profiles of the PHCAMTA genes during abiotic stress

To investigate the expression of PHCAMTA during abiotic stress, we analyzed the expression of 11 PHCAMTAs under three abiotic stresses using qRT-PCR: polyethylene glycol (PEG), heat, and cold treatment. the expression patterns of PHCAMTAs responded differently to the three abiotic stresses, and some PHCAMTAs were either significantly induced or repressed. The expression pattern of most genes changed significantly during the early phase (0-6 h) of the stress response.

As shown in the (Fig 7), the expression of the Mao bamboo CAMTA gene family under drought stress. PHCAMTA gene expression showed weak changes when subjected to drought stress for 3 h, and the expression at 6 h of stress was significantly higher than that at other times of stress. most of the genes, except PHCAMTA 06 and PHCAMTA 10, had a higher expression at 24 h of drought stress than the expression of most of the genes was slightly higher at 24 h of drought stress than at 12 h of drought stress.

In contrast, the overall expression of CAMTA in Moso bamboo was higher when subjected to drought stress than when subjected to cold stress, and it is assumed that PHCMATA is mainly involved in drought stress regulation.

Discussion

Genome-wide identification and phylogenetic analysis of the CAMTA gene of Moso bamboo

CAMTAs are a specific class of plant transcription factors that play an essential role in the regulation of plant growth and development and metabolism (Galon et al. 2008; Yang et al. 2012; Yang & Poovaiah 2002). The molecular functions of CAMTA have been verified not only in Arabidopsis (Galon et al. 2010; Pandey et al. 2013) and rice as model plants, but also in cotton (Pant et al. 2018), maize (Yue et al. 2015), tobacco (Kakar et al. 2018) and tomato (Yang et al. 2012), where the CAMTA gene family has been gradually identified. However, no studies on CAMTAs have been conducted in the economically important bamboo species, moso bamboo.

Currently, the draft genome of Moso bamboo is largely complete, allowing for a full identification of key gene families (Peng et al. 2013; Zhao et al. 2018). We classified the 11 PHCAMTA genes into five categories, ClassI, ClassII, ClassIII and ClassIV, based on phylogenetic analysis. Among them, four members (36%) belonged to Class I, three members (27%) to Class II, three members (27%) to Class III, and one member (9%) to Class IV (Fig 1). Gene structure analysis reveals structural differences among members within the same subfamily. Such as, PHCAMTA members in the same family I have intron numbers ranging from 10-14. Therefore, we hypothesize that members of subfamily I may have undergone pruning of gene fragments during their evolution (Li et al. 2016; Staiger & Brown 2013). Nevertheless, the similar conserved sequences and gene structures among CAMTA family members suggest that gene biological functions are generally the same within a family. All six NTR1 homologs of Arabidopsis have a conserved structural feature with a DNA-binding region (CGCG structural domain) at the N-terminal end and a CaM-binding structural domain at the C-terminal end. The role of Ca^{2+}/CaM may be expressed in controlling interactions with other proteins or altering transcriptional activation of other proteins. In addition, conserved domain comparison showed that all PHCAMTA genes have CG-1 structural domains, indicating that the conserved motifs of the CAMTAs family are broadly conserved during evolution.

During signal transduction, multiple cis-acting elements on a gene promoter work together to regulate multiple complex biological responses. S.lycopersicum SlCAMTA gene contains salt stress regulatory elements, including ABRE, G-box, MBS, and TGA (Wang et al. 2021), and CAMTAs of different species have been reported to respond to a variety of biotic and abiotic stresses, including low temperature, hormones, high salt, and drought. Two genes, ZmCAMTA4 and ZmCAMTA6, were highly expressed under stress treatment, and cis-element analysis revealed the involvement of CAMTA genes in the association between environmental stress and stress-related hormones, and the GhCAMTA gene family may also be involved in the phytohormone signaling pathway (Liu et al. 2021; Pant et al. 2018). On the basis of PlantCARE software, we found that elements involved in abscisic acid response, MeJA response, growth hormone (IAA) and many other hormone regulation-related elements were present. Therefore, we suggest that PHCAMTA genes may also be involved in the stress response of plants. Interestingly, the promoter regions of most PHCAMTA genes have the largest number of MYB elements involved in drought induction (Fig. 5). Previous studies on the response of this family of genes to abiotic stresses are relatively scarce, but recent studies on wheat confirmed that the expression of TaCAMTA1a-B and TaCAMTA1b-B. 1 was down- and up-regulated, respectively, in response to drought stress to maintain normal physiological functions associated with the plant, and wheat CAMTA family members also contain a large number of MYB elements (Wang et al. 2022).

Evolutionary Characterization of the PHCAMTA Family

Gene duplication may produce new genes, which greatly helps in the evolution of gene function. The three evolutionary patterns of gene replication are (Liu et al. 2019): segmental duplication, tandem duplication and translocation events. Segmental and tandem replication are the most

common basis for gene family expansion in plants (Freeling 2009; Li & Barker 2020). Previous studies on whole genome replication have shown that the genome size of Bamboo (2051.7 Mb) and its close relative, Z.mays (2066.4 Mb), is similar, but the number of CAMTA families is higher than that of the latter (Chen et al. 2020). Therefore, we performed a consistency analysis within and among the Moso bamboo genomes. Within the Moso bamboo genome, there was one pair of segmental duplication genes in the CAMTA gene. Therefore, the amplification of the CAMTA gene family mainly comes from gene fragment replication. Simultaneous analysis of the genome of Bamboo and four other sequenced plant genomes showed that the members of the bamboo CAMTA family had significant consistency with the genomes of the monocot plant rice.

The role of CAMTA genes in different tissues and organs

Several studies have shown that CAMTAs can regulate plants during the developmental period of lateral organs, such as important effects on plant organs formation (Rahman et al. 2016; Shangguan et al. 2014; Wang et al. 2015; Yang et al. 2015), which is consistent with our findings. Analysis of expression profiles in different bamboo tissues revealed that a large number of PHCAMTAs showed the amount of expression varies in different tissues (Fig 6). For example, PHCAMTA07 was highly expressed in root tissues, and it is speculated that PHCAMTA07 gene function may be similar to that of NtabCAMTA03 in tobacco, which is directly involved in stem tip meristem tissue production for differentiation into leaf primordia. Interestingly, the expression of some PHCAMTAs in leaves is higher than in flowers, and it is speculated that they are mainly involved in the plant growth process but not in the process of plant flower bud differentiation.

Expression of PHCAMTA Genes in Responses to Cold, Drought and Heat Treatments

The PheE2F/DP promoter in response to drought stress contains many MYB and MYC2 binding sites (Li et al. 2021). The involvement of PHCAMTA in drought stress regulation was also confirmed in subsequent expression analyses, which revealed a large number of MYB and MYC elements in PHCAMTA regulated by drought stress. We found that the expression levels of all genes increased overall at the beginning of abiotic stress in Moso bamboo, and decreased to the lowest expression level at 24h of stress. The expression level showed an increasing trend at 12h-24h of drought stress, and it was speculated that Moso bamboo responded to drought stress after 12h of stress in order to enhance its stress resistance. Our study suggests that the PHCAMTA gene family plays an essential role during drought stress response, but more studies are needed to reveal the functional significance of the CAMTA gene family in moso bamboo.

Conclusions

Our results present new findings for the moso bamboo CAMTA family and provide partial experimental evidence for further validation of the function of PHCAMTAs.

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Table 1(on next page)

Physicochemical properties of proteins encoded by CAMTA genes in Phyllostachy edulis

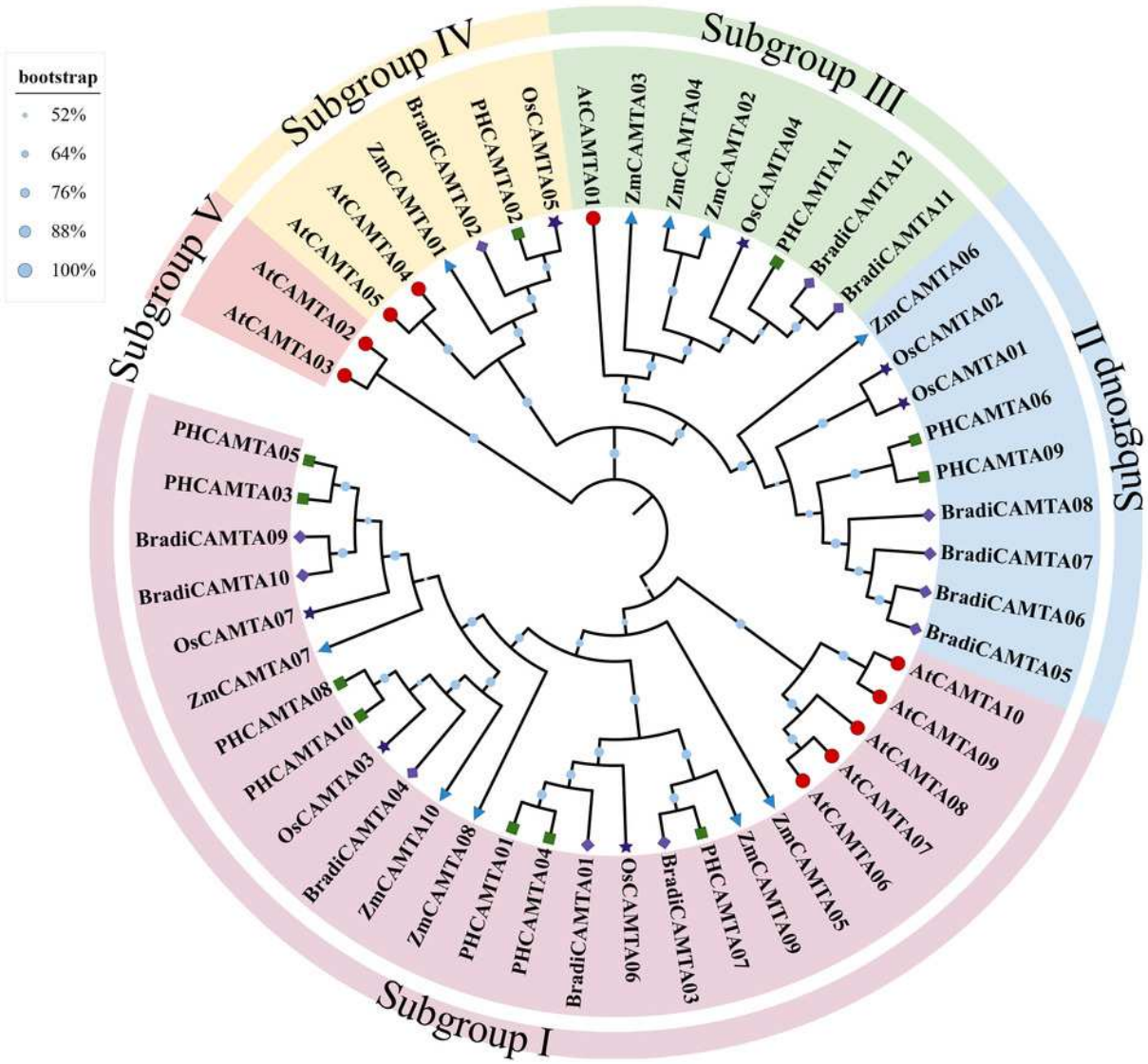
Table 1 Physicochemical properties of proteins encoded by CAMTA genes in Phyllostachy edulis

ID	Gene name	Number of amino acids	Molecular weight （kDa）	Theoretical pI	Aliphatic index	Grand average of hydropathicity (GRAVY)	signal peptide
PH02Gene18220.t1	*PHCAMTA01*	1024	113.88	5.74	77.09	-0.456	NO
PH02Gene42704.t1	*PHCAMTA02*	925	103.43	8.2	76.1	-0.477	N
PH02Gene37813.t1	*PHCAMTA03*	1027	114.18	5.51	74.14	-0.503	N
PH02Gene40726.t1	*PHCAMTA04*	1030	114.81	5.49	75.5	-0.507	N
PH02Gene36566.t1	*PHCAMTA05*	816	90.10	5.18	74.73	-0.45	N
PH02Gene07259.t1	*PHCAMTA06*	851	96.14	7.61	80.82	-0.47	N
PH02Gene08544.t1	*PHCAMTA07*	1028	114.92	5.69	77.72	-0.48	N
PH02Gene05448.t1	*PHCAMTA08*	1025	114.11	5.92	76.92	-0.50	N
PH02Gene05785.t1	*PHCAMTA09*	851	96.18	6.51	77.39	-0.55	N
PH02Gene15267.t1	*PHCAMTA10*	1026	114.85	5.78	75.30	-0.51	N
PH02Gene16049.t4	*PHCAMTA11*	1031	114.95	5.88	74.03	-0.567	N

Figure 1

Phylogenetic tree analysis of CAMTA sequences.

The full-length amino acid sequences of 50 CAMTA proteins were used to construct the phylogenetic tree using MEGA7.0 with the neighbor-joining (NJ) method. The size of graphics at the branch represents the confidence relative value obtained by 100 bootstrap tests. AtCAMTA represents CAMTA protein sequence of Arabidopsis thaliana, OsCAMTA represents CAMTA protein sequence of rice, ZmCAMTA represents CAMTA protein sequence of maize and BradiCAMTA represents CAMTA protein sequence of Brachypodium distachyon.


bootstrap
52%
64%
76%
88%
100%
Subgroup IV
Subgroup III
Subgroup V
Subgroup II
Subgroup I
AtCAMTA01
ZmCAMTA03
ZmCAMTA04
ZmCAMTA02
OsCAMTA04
PHCAMTA11
BradiCAMTA12
BradiCAMTA11
OsCAMTA05
PHCAMTA02
BradiCAMTA02
ZmCAMTA01
AtCAMTA04
AtCAMTA05
AtCAMTA02
AtCAMTA03
ZmCAMTA06
OsCAMTA02
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PHCAMTA09
BradiCAMTA08
BradiCAMTA07
BradiCAMTA06
BradiCAMTA05
AtCAMTA10
AtCAMTA09
AtCAMTA08
AtCAMTA07
AtCAMTA06
ZmCAMTA05
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OsCAMTA06
BradiCAMTA01
PHCAMTA04
PHCAMTA01
ZmCAMTA08
ZmCAMTA10
BradiCAMTA04
OsCAMTA03
PHCAMTA10
PHCAMTA08
ZmCAMTA07
OsCAMTA07
BradiCAMTA10
BradiCAMTA09
PHCAMTA03
PHCAMTA05

Figure 2

Gene structure, conserved motifs and conserved domains of PHCAMTA.

Phylogenetic trees were made with maximum likelihood by using the Neighbor joining model and MEGA 7.0 software. Different colors plates represent different groups. (A) Exon–intron distribution of PHCAMTA. (B) Conserved motifs in PHCAMTA. Motif 1 to motif 8 represented different motifs, and they were represented by different color boxes on the right. (C) Conserved domains in PHCAMTA. CG-1, CG-1 domains. TIG, IPT/TIG domain. ANKYR, ankyrin repeats. IQ, is a calmodulin-binding motif.

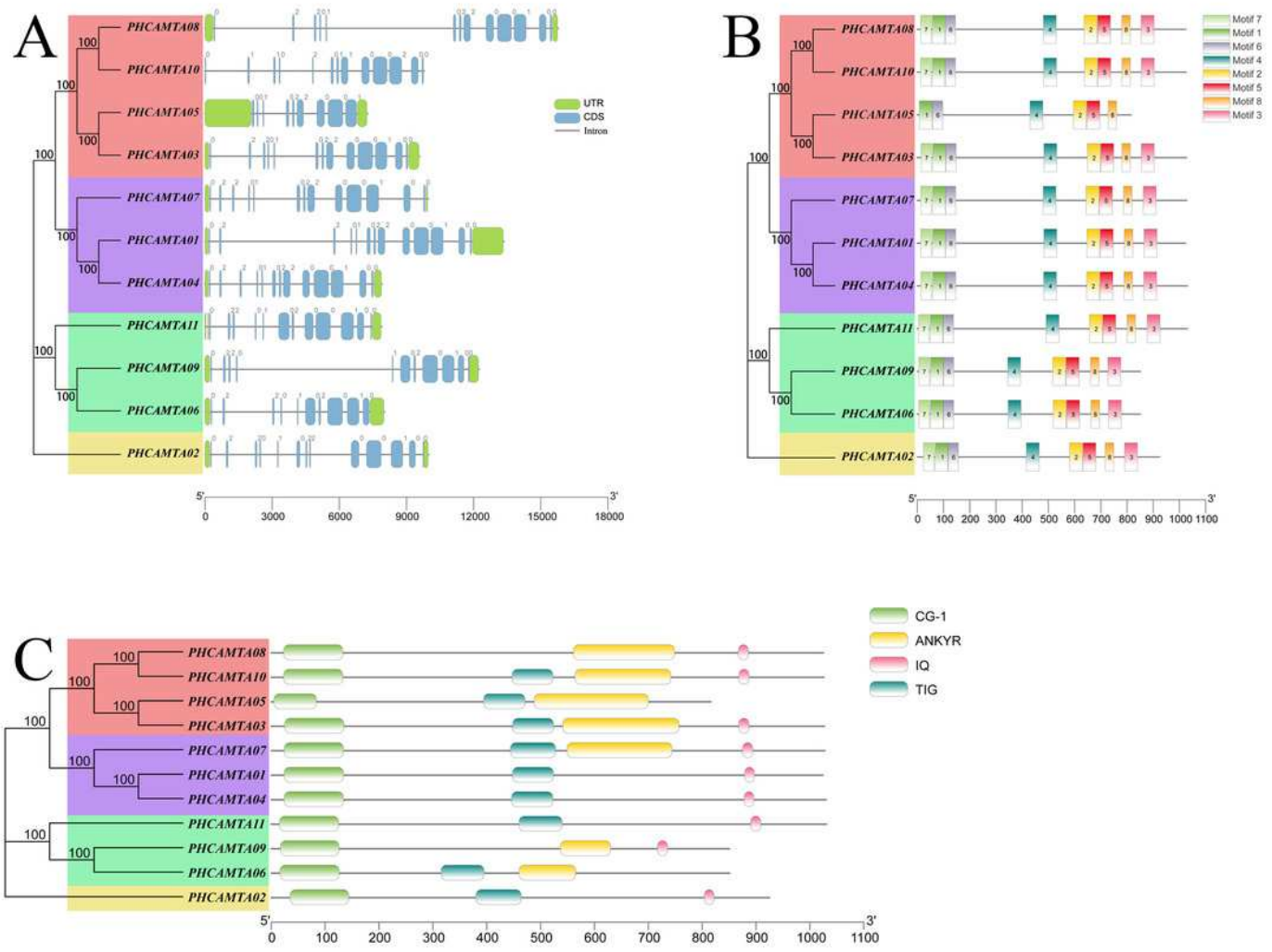

Figure 3

The distribution and duplication events of PHCAMTA on the chroMosome.

The location of these genes on the chroMosome was visualized using the visualization tools.

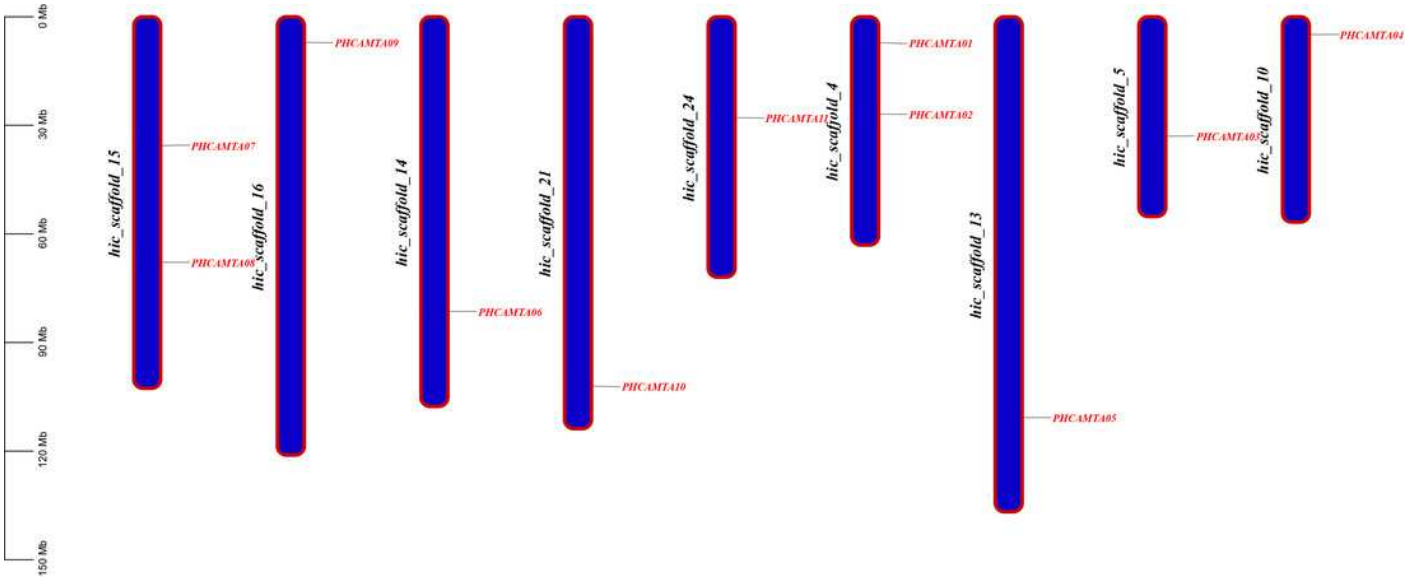

Figure 4

synteny relationships (A) Intraspecific colinearity analysis.

A total of 11 PHCAMTAs were mapped onto the chroMosomes on the basis of their physical location. ChroMosome numbers (scaffold1-scaffold24) are distributed in the outer circle, the red lines indicate duplicated PHCAMTA gene pairs. (B) Analysis of collinearity between different species. The gray lines indicate duplicated blocks, while the red lines indicate duplicated PHCAMTA gene pairs. ChroMosome numbers are at the bottom of each chroMosome.

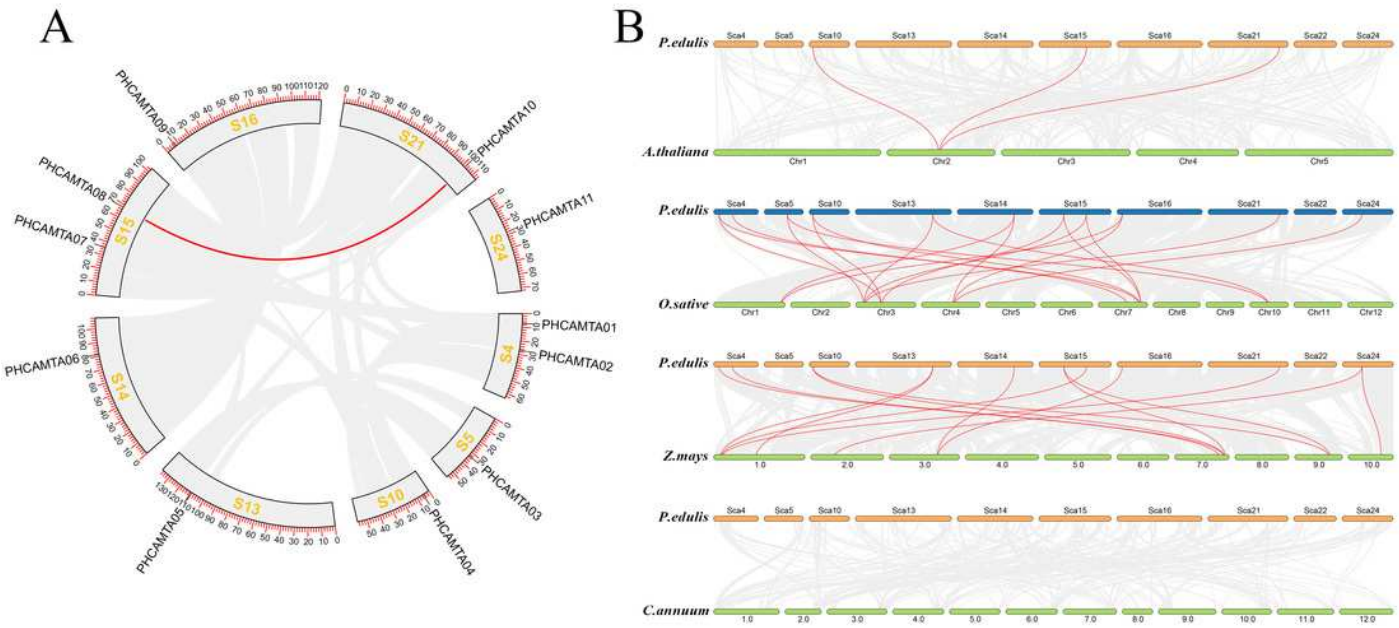

Figure 5

Cis-acting elements in PHCAMTA promoters.

The circle represented the number of specific cis-acting elements per gene. The chart and number on the right indicated the number of genes corresponding to the specific cis-acting element.

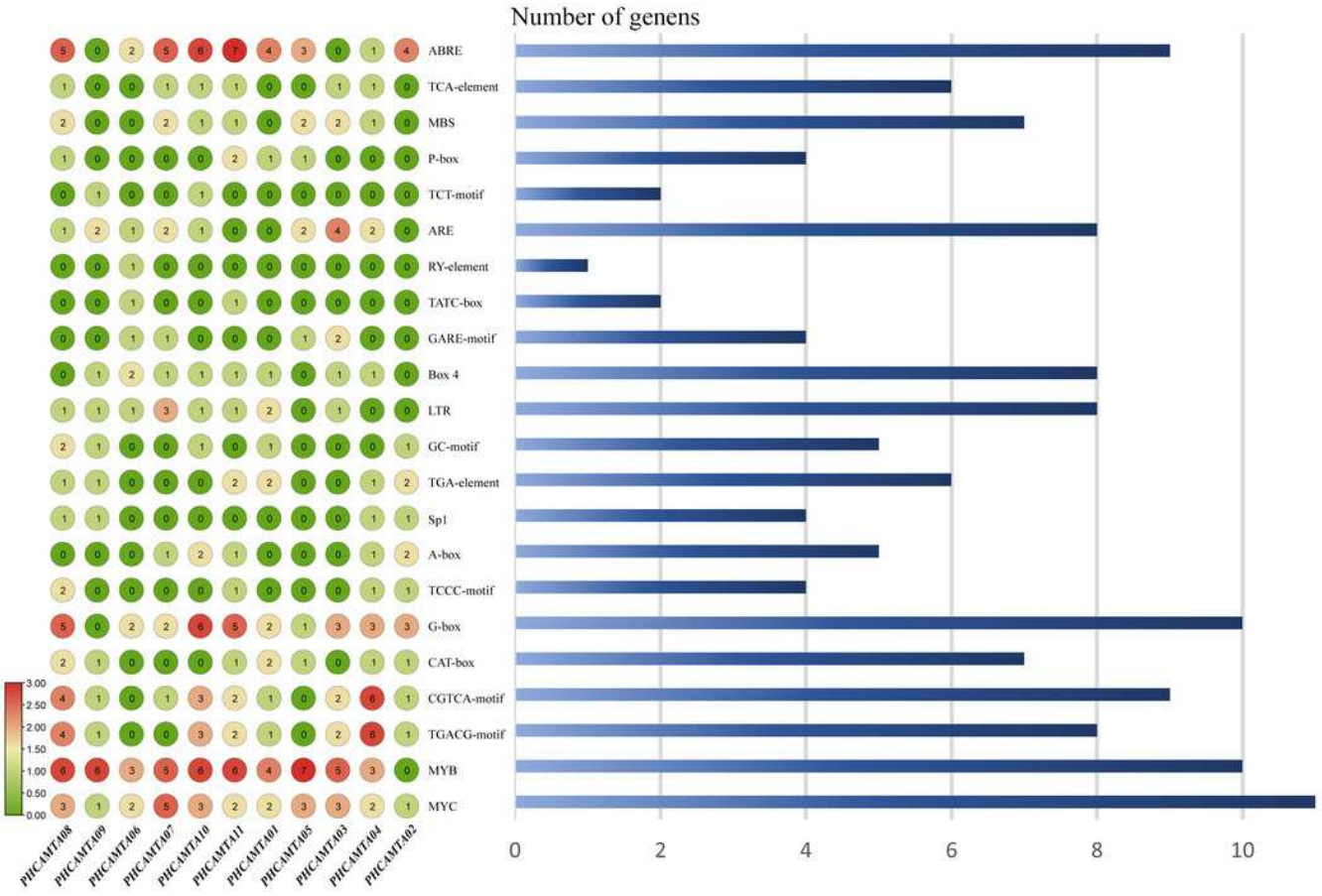

Figure 6

Expression profile cluster analysis of PHCAMTA genes with differential tissue expression.

Heatmap showing relative expression levels of PHCAMTAs in roots, leaves, panicles, and rhizomes. The values are expressed with log2 TPM.

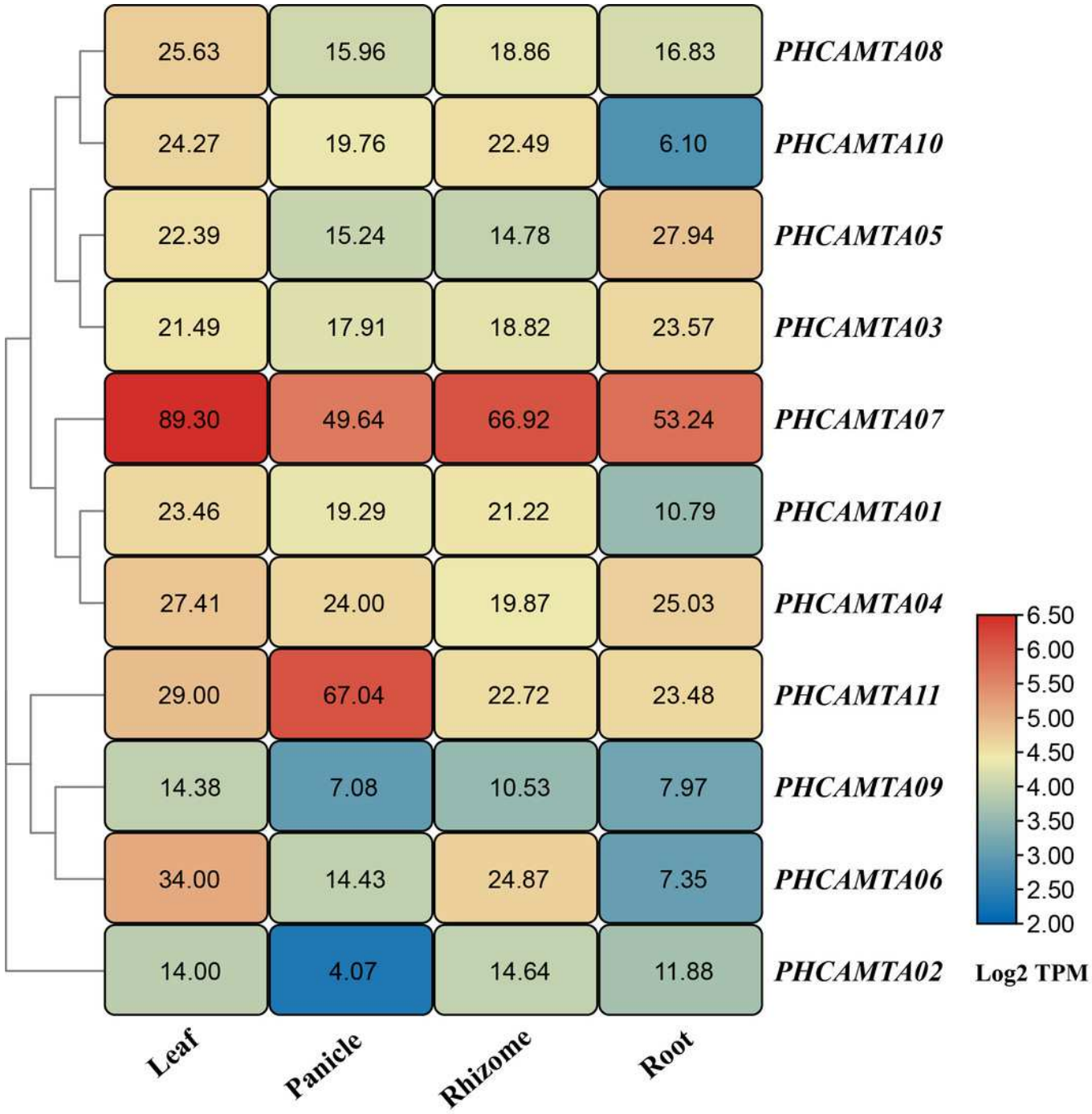
25.63
15.96
18.86
16.83
PHCAMTA08
24.27
19.76
22.49
6.10
PHCAMTA10
22.39
15.24
14.78
27.94
PHCAMTA05
21.49
17.91
18.82
23.57
PHCAMTA03
89.30
49.64
66.92
53.24
PHCAMTA07
23.46
19.29
21.22
10.79
PHCAMTA01
27.41
24.00
19.87
25.03
PHCAMTA04
29.00
67.04
22.72
23.48
PHCAMTA11
14.38
7.08
10.53
7.97
PHCAMTA09
34.00
14.43
24.87
7.35
PHCAMTA06
14.00
4.07
14.64
11.88
PHCAMTA02
Leaf
Panicle
Rhizome
Root
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
Log2 TPM

Figure 7

Results of RT-qPCR analysis showing the expression patterns of CAMTA genes in Moso bamboo subjected to abioic stress at different time points.

A: Expression of 11 PHCAMTA genes under three abiotic stresses; A1: cold stress; A2: heat stress; A3: drought stress. B: Comparison of PHCAMTA gene expression under three abiotic stresses.

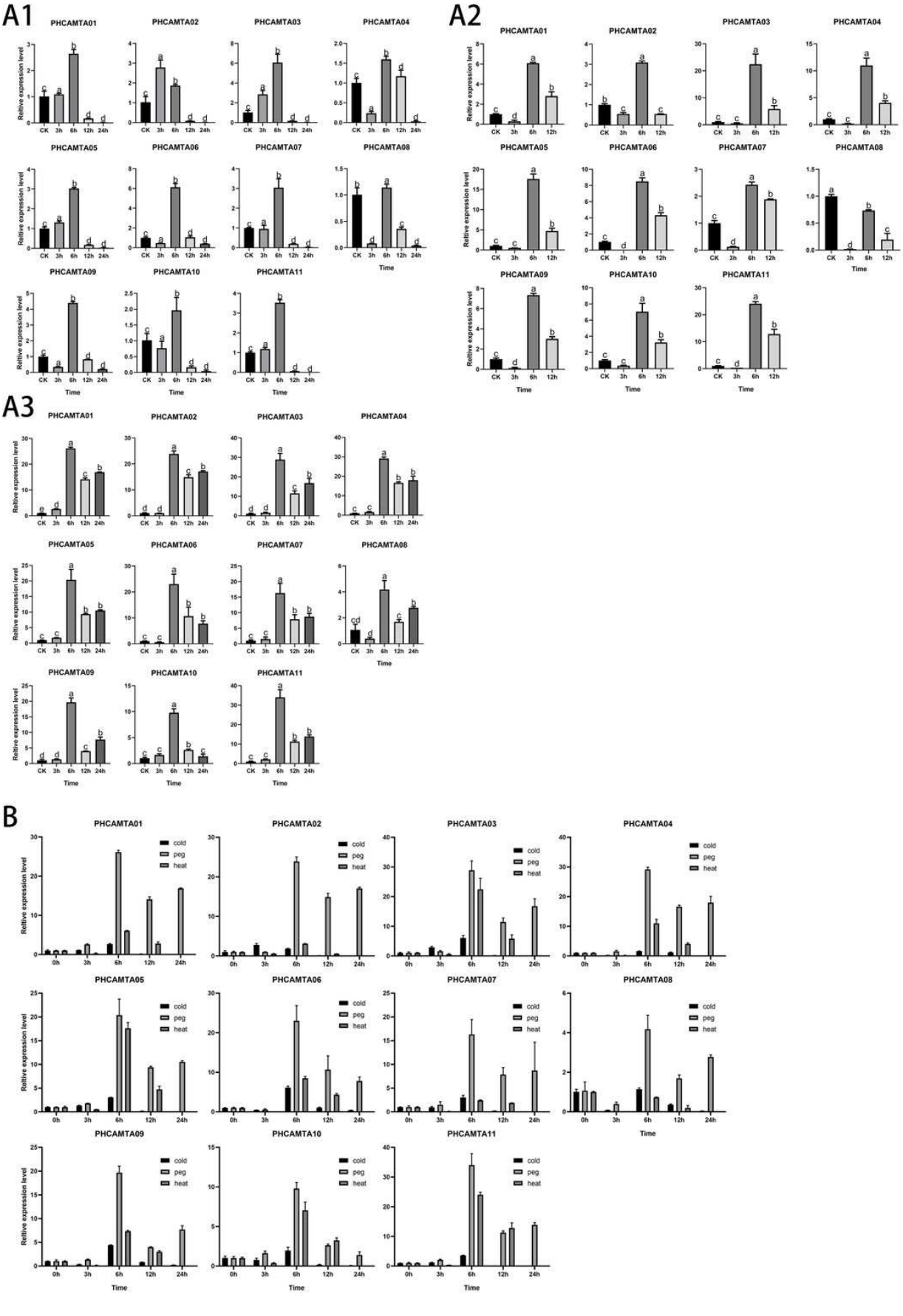
A1
PHCAMTA01
PHCAMTA02
PHCAMTA03
PHCAMTA04
PHCAMTA05
PHCAMTA06
PHCAMTA07
PHCAMTA08
PHCAMTA09
PHCAMTA10
PHCAMTA11
Reltive expression level
CK 3h 6h 12h 24h
Time
A2
PHCAMTA01
PHCAMTA02
PHCAMTA03
PHCAMTA04
PHCAMTA05
PHCAMTA06
PHCAMTA07
PHCAMTA08
PHCAMTA09
PHCAMTA10
PHCAMTA11
Reltive expression level
CK 3h 6h 12h
Time
A3
PHCAMTA01
PHCAMTA02
PHCAMTA03
PHCAMTA04
PHCAMTA05
PHCAMTA06
PHCAMTA07
PHCAMTA08
PHCAMTA09
PHCAMTA10
PHCAMTA11
Reltive expression level
CK 3h 6h 12h 24h
Time
B
PHCAMTA01
PHCAMTA02
PHCAMTA03
PHCAMTA04
PHCAMTA05
PHCAMTA06
PHCAMTA07
PHCAMTA08
PHCAMTA09
PHCAMTA10
PHCAMTA11
cold
peg
heat
Reltive expression level
0h 3h 6h 12h 24h
Time

Figure 8

Results of RT-qPCR analysis showing the expression patterns of CAMTA genes in Moso bamboo subjected to abioic stress at different time points.

C: Comparison among PHCAMTA genes in Moso bamboo when subjected to abiotic stress.

Bars with same letter means no significant difference based on LSD test ($p \leq 0.05$).