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Soybean Calmodulin-binding Transcription Activators, GmCAMTAs, Negatively Coordinate the Circadian Regulation of Developmental Processes and Drought Stress Responses in Transgenic *Arabidopsis*

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Abstract: The calmodulin-binding transcription activators (CAMTAs) mediate transcriptional regulation of development, growth, and responses to various environmental stresses in plants. To characterize the biological processes of soybean CAMTA (*GmCAMTA*) family members in response to abiotic stress, we identified 15 *GmCAMTA* genes from soybean (*Glycine max* L.) and investigated their roles in the abiotic stress response. The transcriptions of *GmCAMTAs* exhibited distinct circadian regulation patterns and were expressed differently in response to salt, drought, and cold stresses, except ABA. Interestingly, the expression levels of *GmCAMTA2*, *GmCAMTA8*, and *GmCAMTA12* were higher in stem tissue than in other soybean tissues. To determine the roles of *GmCAMTAs* in the regulation of developmental processes and stress responses, we isolated *GmCAMTA2* and *GmCAMTA8* cDNAs from soybean and generated *Arabidopsis* overexpressing transgenic plants. The *GmCAMTA2*-OX and *GmCAMTA8*-OX plants showed hypersensitivity to drought stress. The water in the leaves of *GmCAMTA2*-OX and *GmCAMTA8*-OX plants was lost faster than that in WT plants under drought stress conditions. In addition, stress-responsive genes in the *GmCAMTA2*-OX and *GmCAMTA8*-OX plants were down-regulated under drought stress conditions. Our results suggest that *GmCAMTA2* and *GmCAMTA8* genes are regulated by circadian rhythms and function as negative regulators in development and drought stress responses.

Keywords: GmCAMTA; circadian rhythm; development; drought stress

1. Introduction

In plants, the calcium (Ca²⁺) ion is an important element for maintaining the intracellular ion homeostasis of plant cells, as well as for regulating structural processes, metabolism, and signaling [1,2]. The major function of the Ca²⁺ ion is to enhance the plant's tolerance to stabilize cell walls and membranes against various plant stresses [2–4]. The Ca²⁺ ion regulates the biochemical reactions in the plant cell by controlling the activity of ion-channel-related proteins, such as cyclic nucleotide-gated channels (CNGCs), ionotropic glutamate receptors, two-pore channel 1 (TPC1), annexins, and mechanosensitive channels [2]. The Ca²⁺ ion is a versatile secondary messenger in biotic and abiotic

stress responses [3,4]. Increased Ca^{2+} concentrations through various abiotic stresses are recognized, amplified, and transmitted through Ca^{2+} -binding proteins [4]. These Ca^{2+} -binding proteins play a role as Ca^{2+} sensors, including calmodulin (CaM) and calmodulin-like proteins (CMLs), calcineurin B-like proteins (CBLs), and calcium-dependent protein kinases (CDPKs) [5]. These signal cascades in plants physiologically regulate the division and elongation of cells, stomatal movement, and developmental processes in plant tolerance or adaptation responses to abiotic stresses [5,6].

The CaMs with one or more EF-hand Ca^{2+} -binding motifs are associated with a multitude of biochemical reactions, such as the control of transcriptional expression and enzyme activity [6,7]. Although the Ca^{2+} ion lacks cellular levels to activate CaM under natural conditions, when exposed to abiotic stresses, the Ca^{2+} levels are rapidly increased, generating the Ca^{2+} signals, and there is a boost in the activity of CaM [8]. CaM is the best characterized of the Ca^{2+} receptors, and the Ca^{2+} -CaM complex regulates the ion channels, transporters, transcription factors (TFs), and various enzymes, including protein kinase, phosphatases, and metabolic enzymes [9,10]. The CaM-driven TFs such as calmodulin-binding transcription activators (CAMTAs), DREB, MYB, WRKY, NAC, bZIP, bHLH, and MADS-box play a pivotal role in multiple cellular processes, including plant stress responses and developmental processes [5,10].

A total of 465 CAMTA genes have been identified from 112 plant species, including dicots, monocots, basal angiosperms, gymnosperms, lycophyta, monilophytes, bryophytes, and chlorophytes [10]. Of the total, 302 CAMTAs were obtained from 49 dicots among plant species; in addition, 15 CAMTAs are the best isolated from soybean (*Glycine max* L.), respectively [10]. CAMTA proteins in soybean have been known to comprise conserved functional domains, including the DNA-binding (CG-1) domain, transcription-associated immunoglobulin (TIG) domain, calmodulin-binding (CaMB) domain, ankyrin (ANK) repeats, and IQ motifs [11]. The CG-1 domain recognizes and binds to specific DNA sequences of the CGCG box for activating gene transcription [12,13]. The ANK repeats and the TIG domain with binding to the non-specific DNA are involved in protein-protein interactions with either homo- or heterodimers [12,14]. A diverse number of IQ motifs in the C-terminal regions of CAMTAs contain amino acid sequences, such as IQXXRGXXX, as Ca^{2+} -independent calmodulin-binding (CaMB) sites [5,12,15]. The CaMB domain with its α -helix structure is an important Ca^{2+} -dependent CaMB domain for determining characteristics of CAMTAs in plants [12,16].

The expression levels of CAMTAs show significant differences in the extensive developmental stages and plant stress responses [5,10]. In *Arabidopsis*, the expression of *AtCAMTA6* (AT3G16940) shows a high level in mature leaves and siliques compared with that in other tissues [10]. In addition, the expression levels of *AtCAMTA2* (AT5G64220) in seeds and young leaves are significantly lower than in other tissues [10]. In tomato (*Solanum lycopersicum*), *PtCAMTA4* (JN566050) shows high levels of transcriptional expression in turning and orange fruit stages, while *PtCAMTA2* (JN566047) is highly expressed in all developmental fruit stages except the mature green and breaker stages [16]. In tea (*Camellia sinensis*) plant, the expression of *CsCAMTA1* (TEA025813.1) is at higher levels in old leaves than that in both other tissues and other *CsCAMTA* genes [17]. In black cottonwood (*Populus trichocarpa*), *PtCAMTA1* (POPTR_0001s13700) is highly expressed in the mature leaves, while *PtCAMTA2* (POPTR_0005s07660) and *PtCAMTA3* (POPTR_0007s05410) are more weakly expressed in the roots than in different tissues [18]. In rice (*Oryza sativa*), the expression levels of all seven *OsCAMTA* genes are displayed more in leaves after flowering compared with those before flowering [10]. The *MuCAMTA1* (*Musa acuminata*; LOC1039776) and *phavuCAMTA1* (*Phaseolus vulgaris*; PHAVU_006G206400g) genes are highly expressed in plant responses under drought stress conditions [19,20]. In the root of maize (*Zea mays* L.), the expression of *ZmCAMTA1* (GRMZM2G171600) is significantly decreased to cold stress, but increases to salt stress and jasmonic acid (JA) treatment [21]. In *Medicago truncatula*, the expression of *MtCAMTA7* (Medtr8g090205) is induced by ABA treatment compared with other *MtCAMTA* genes [22]. Therefore, the expression of CAMTA genes presents substantial specificity in the developmental stages and tissues, as well as in stress responses.

CAMTAs in plants are known to be positive or negative regulators in plant development processes, as well as in biotic and abiotic stress responses [23–25]. *AtCAMTA1* (*AT5G09410*) positively regulates the ABA-dependent response for enhancing plant tolerance to drought stress [23]. *atcamta1* mutants showed hypersensitivity to drought stress by regulating stress-responsive genes, such as *RD26*, *ERD7*, *RAB18*, *LTPs*, and *COR78* [23]. *AtCAMTA3* (*AT2G22300*) functions as a negative regulator in plant defense responses against bacteria [26] and fungi [27]. *atcamta3* mutants showed enhanced resistance to *Pseudomonas syringae* pv. *tomato* DC3000 (*PstDC3000*) and *Botrytis cinerea* by the high expression of *pathogenesis-related* (*PR*) genes [26,27]. In contrast, *AtCAMTA3* acts as a positive regulator in plant tolerance to freezing stress [24]. A mutation of *atcamta3* weakened the freezing tolerance caused by reducing the cold-induced accumulation of *CBF* genes transcript levels during exposure to low temperature [24]. *AtCAMTA1* and *AtCAMTA5* (*AT4G16150*) positively regulate the pollen developmental process by increasing the expression of *Arabidopsis V-PPase 1* (*AVP1*) [28]. Transcriptome profiling using a *camta6* mutant under salt stress showed that 638 up-regulated and 1,242 down-regulated genes were involved in *AtCAMTA6*-dependent manners [29]. In rice, *OsCBT/OsCAMTA5* (*LOC_Os07g30774*) plays a negative role in the plant defense response against fungal pathogens by the high expression of *PR* genes, such as *PR1*, *PR4*, *PR10*, and *PBZ* [30]. Rice *oscbt-1* mutants showed enhanced plant disease resistance against both rice blast fungus (*Magnaporthe grisea*) and bacteria (*Xanthomonas oryzae*) [30]. Soybean *GmCAMTA12* (*Glyma.17G031900*) regulated the expression of its regulatory network-related genes with the *CGCG/CGTTG* motif under drought stress in *Arabidopsis* and soybean hairy roots [31]. In peach (*Prunus persica* L. Batsch), *PpCAMTA1* (*Prupe.1G108700*) in *atcamta2/3* double mutants suppressed salicylic acid (SA) biosynthesis and the expression of SA-related genes in the plant defense response against *PstDC3000* [32]. Thus, CAMTAs in plants play crucial roles as transcription factors in both biotic and abiotic stress responses.

Various environmental changes have many implications for growth, developmental processes, and yield in crop plants. Drought stress is an important restricting factor for crop growth and the developmental tissue processes, holding both expansion and elongation down [33]. Since drought stress triggers low turgor pressure in the cell, the osmotic maintenance of cell turgor in crops is of great importance for plant growth and crop survival [33]. In many crops, soybean is highly sensitive to drought stress responses through various morphological changes [34]. When exposed to drought stress, soybean shows momentous changes in most of the tissue morphology, such as a reduction in new branches and trifoliate leaves per plant, growth inhibition of initiation leaves, and reductions in leaf area via repression of lamina expansion [34]. Drought stress has a negative effect on the developmental processes of stems and leaves by decreasing their elongation and expansion [33].

Soybean (*Glycine max* L.) is one of the most economical crops with a nutritional balance of protein, fat, and carbohydrate. It is mainly used as a portion in important foods, feeds, and bio-feedstocks [35]. The Williams 82 cultivar is a typical soybean breed that was used to be established the reference genome sequence [36]. The 15 *GmCAMTAs* were identified in soybean by the SoyBase database (<http://soybase.org/>), and the analysis of gene expression showed that the expression patterns of *GmCAMTAs* were altered during abiotic stress (salt, drought, cold, and oxidative) and hormone (ABA, SA, and JA) responses (Supplementary Table S1) [11]. Recently, it was reported that *GmCAMTA12* plays an important role in the tolerance responses of *Arabidopsis* and soybean to drought stress [31]. However, *GmCAMTAs* still do not have enough study for the functional characterization in plant development processes and stress tolerance responses. In this study, *GmCAMTA2* and *GmCAMTA8* characterized the substantial specificity and reliability in the developmental stages and abiotic stress responses. The results of our study can be effective for further functional analysis of *GmCAMTAs* in soybean.

2. Results

2.1. Circadian rhythms affected the expression patterns of *GmCAMTAs* under long-day conditions

The stress-responsive genes in plants are rhythmically expressed in stress signaling, thereby producing daily cellular, metabolic, and physiological rhythms [37]. To investigate the influence on

the transcripts of *GmCAMTAs* by daily circadian rhythms, the expression patterns of *GmCAMTAs* under long-day conditions (16h light/8h dark) were measured. Quantitative RT-PCR analyses showed that the expression of 15 *GmCAMTAs* accumulated with the onset of light and reached maximum access levels of their expression at ZT9 to ZT15 (Figure 1). After the light was turned off (ZT18 to ZT20), the expression levels of most *GmCAMTAs* significantly decreased more than approximately two-fold under light conditions (Figure 1). However, the expression levels of *GmCAMTA3*, *GmCAMTA5*, *GmCAMTA6*, *GmCAMTA7*, and *GmCAMTA13* at ZT18 and ZT20 were subtly reduced less than two-fold than between ZT9 and ZT15 (Figure 1). In addition, *in silico* analysis using the Diurnal database tool showed the diurnal expression of *GmCAMTA2*, *GmCAMTA5*, *GmCAMTA8*, *GmCAMTA12*, and *GmCAMTA13* (Supplementary Figure S1). Unfortunately, the diurnal expression changes of other *GmCAMTAs* were not available in the Diurnal web database. Therefore, the transcripts of *GmCAMTAs* were regulated according to circadian rhythms in the daily clock.

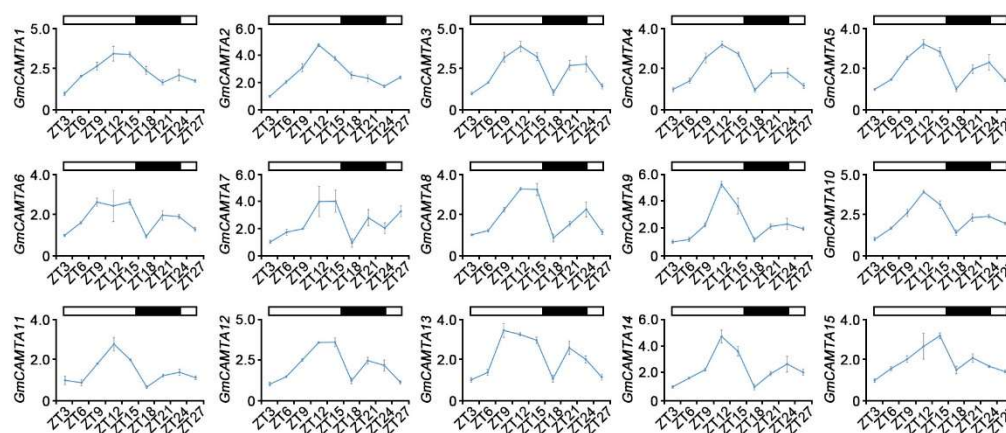


Figure 1. Transcriptional influence of *GmCAMTAs* in circadian rhythms. The expression levels of *GmCAMTAs* were analyzed for circadian regulation using qRT-PCR. The two-week-old seedlings of wild-type (WT) soybeans (*Glycine max* cv. Williams 82) were grown under long-day conditions (16h light / 8h dark). Zeitgeber time (ZT) means a time of turning on a light. The white and black areas mark the approximate division of light and dark. qRT-PCR analysis was performed with each *GmCAMTA*-specific primer (Supplementary Table S2). *GmTUBULIN* was used as an internal control for normalization. Error bars represent the SD of three biological replicates, each with three technical replicates.

2.2. Various abiotic stresses affected the expression of *GmCAMTAs*

Previously, a few studies reported that the expression of *GmCAMTAs* is regulated by abiotic stresses and hormone treatment in soybean [11,31]. However, previous results had not considered the rhythmical changes of *GmCAMTAs* expression in abiotic stress responses. To investigate the expression patterns of *GmCAMTAs* under abiotic stress conditions in view of the rhythmical changes, we performed quantitative RT-PCR (qRT-PCR) analysis. After measuring the relative expression values of genes by *GmTUBULIN* as an internal control under abiotic stress conditions, we carried out the normalization according to the relative expression values of genes at each time point under natural conditions. When soybean plants were exposed to salt stress, all of the *GmCAMTAs* were highly expressed in early times, and their expression levels increased gradually as time passed (Figure 2A). The expression levels of most *GmCAMTAs* in drought stress responses were significantly increased compared to those under natural conditions, except *GmCAMTA13* and *GmCAMTA15* (Figure 2B). Interestingly, *GmCAMTA1* and *GmCAMTA2* were highly expressed to cold stress; however, the expression levels of other *GmCAMTAs* were reduced during cold stress responses (Figure 2C). ABA did not influence the expression of most *GmCAMTAs* in soybean, although

GmCAMTA1, *GmCAMTA5*, *GmCAMTA6*, and *GmCAMTA13* were weakly expressed under ABA stress (Figure 2D). Thus, even if *GmCAMTAs* seem invariably to be reactions to most abiotic stresses, the transcripts of *GmCAMTAs* were influenced differently by the specificity of plant stresses.

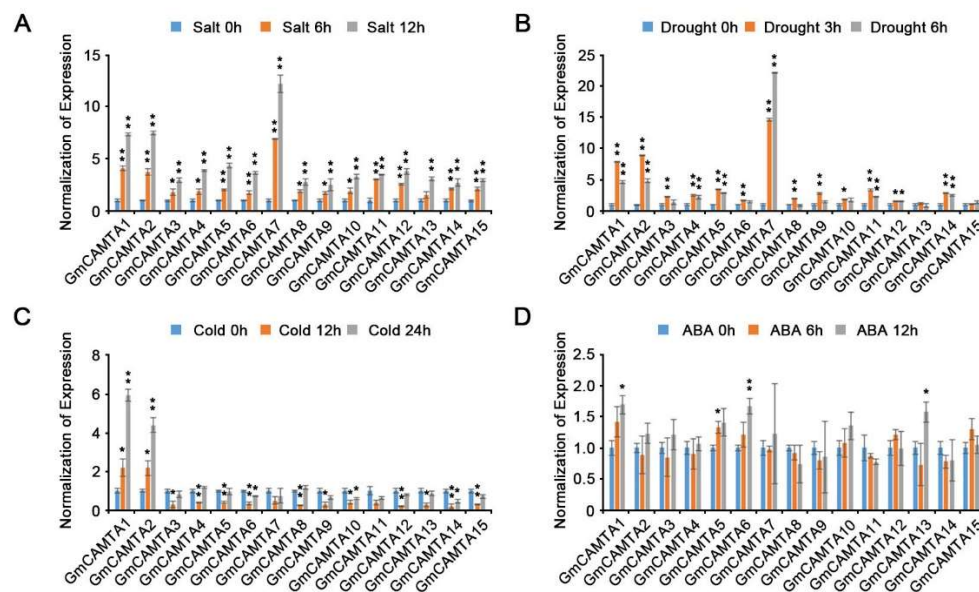


Figure 2. Effects of various abiotic stresses on the transcriptional expression of *GmCAMTAs*. The expression levels of *GmCAMTAs* were analyzed in soybean plants during exposure to various abiotic stresses, using qRT-PCR. The total RNA was extracted from two-week-old plants of WT soybeans under different stresses: **(A)** salt (150 mM NaCl), **(B)** drought, **(C)** cold (approximately 2 °C), and **(D)** 100 μM ABA. qRT-PCR analysis was performed with each *GmCAMTA*-specific primer (Supplementary Table S2). *GmTUBULIN* was used as an internal control, and then the values of the expression levels were calculated by expression values of *GmCAMTAs* under natural conditions for normalization. Error bars represent the SD of three biological replicates, each with three technical replicates. Asterisks indicate significant differences compared with the expression of the zero indicated time point by Student's t-test (* p -value < 0.05, ** p -value < 0.01).

2.3. The transcripts of *GmCAMTAs* had substantial specificity in developmental processes

Plant developmental processes are associated with abiotic stress responses through impressive biochemical, physiological, and morphological changes [38]. We tested the changes in *GmCAMTA* expression levels in different tissues using qRT-PCR analysis. Many *GmCAMTAs* were expressed in the roots; however, *GmCAMTA2*, *GmCAMTA7*, *GmCAMTA9*, *GmCAMTA14*, and *GmCAMTA15* were weakly expressed (Figure 3A). In the leaves, the expression of most *GmCAMTAs* was highly induced, except for that of *GmCAMTA2*, *GmCAMTA8*, and *GmCAMTA12* (Figure 3B). Interestingly, *GmCAMTA2*, *GmCAMTA8*, and *GmCAMTA12* were relatively highly expressed in the stem tissues as compared to other *GmCAMTAs* (Figure 3C). Unlike the expression patterns of *GmCAMTAs* in roots, leaves, and stem tissues, the expression levels of *GmCAMTAs* were similar in the flower (Figure 3D). Our results indicated that *GmCAMTAs* were selectively expressed in each of the plant tissues, thereby showing substantial specificity in the developmental stages.

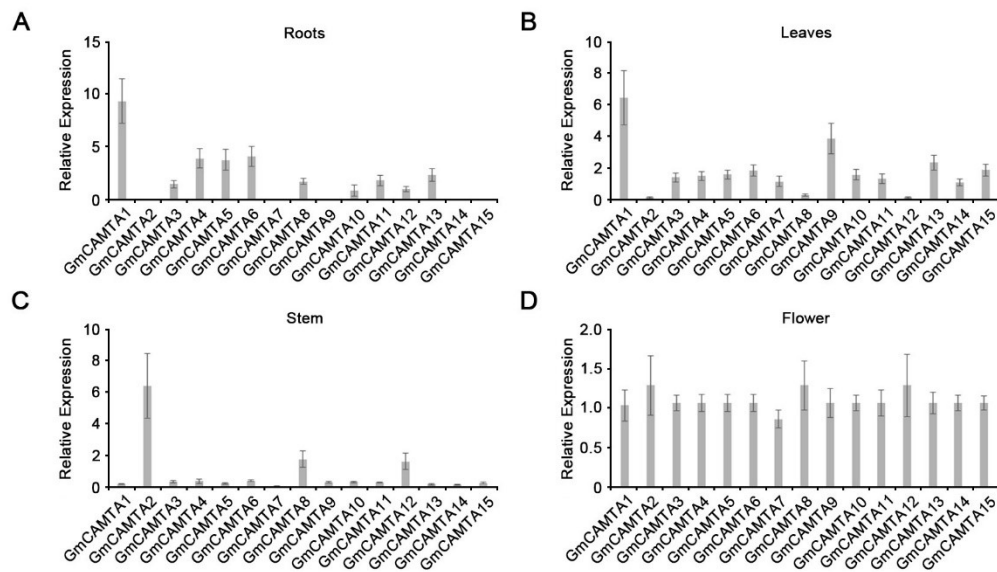


Figure 3. Transcriptional expression of *GmCAMTAs* in soybean tissues. The expression levels of *GmCAMTAs* were analyzed in different tissues of soybean plants using qRT-PCR. The total RNA was extracted from various tissues, including (A) roots, (B) leaves, (C) stem, and (D) flower, at the V4 stages of WT soybeans grown under long-day conditions (16h light / 8h dark). qRT-PCR analysis was performed with each *GmCAMTA*-specific primer (Supplementary Table S2). *GmTUBULIN* was used as an internal control for normalization. Error bars represent the SD of three biological replicates, each with three technical replicates.

2.4. Overexpression of *GmCAMTA2* and *GmCAMTA8* reduced drought tolerance in *Arabidopsis*

GmCAMTA2, *GmCAMTA8*, and *GmCAMTA12* were highly expressed in stem (Figure 3). The overexpression of *GmCAMTA12* in *Arabidopsis* and soybean enhances plant tolerance to drought stress [31]. To investigate the functions of *GmCAMTA2* and *GmCAMTA8* in drought stress responses, we generated *Arabidopsis* transgenic plants with overexpressed *GmCAMTA2* and *GmCAMTA8* under the 35S promoter. To establish the expression levels of *GmCAMTA2* and *GmCAMTA8* in *Arabidopsis* transgenic plants, we performed RT-PCR analysis. According to the results of RT-PCR, we selected three individual lines, each with different expression levels of *GmCAMTA2* (*GmCAMTA2*-OX #1, #4, and #8 lines; Supplementary Figure S2A) and *GmCAMTA8* (*GmCAMTA8*-OX #5, #8, and #9 lines; Supplementary Figure S2B). We examined the plant tolerance by overexpressing *GmCAMTA2* and *GmCAMTA8* during exposure to drought stress (Figure 4). In natural conditions, *GmCAMTA2*-OX and *GmCAMTA8*-OX plants were similar in growth and development compared with WT and empty vector-overexpressing (Vector-OX) *Arabidopsis* (Figure 4A). *GmCAMTA2*-OX and *GmCAMTA8*-OX plants showed hypersensitivity compared with WT and Vector-OX under drought stress and re-watering conditions (Figure 4A). The survival rates after re-watering under drought stress conditions were lower in the *GmCAMTA2*-OX (approximately 2.78 to 13.89 %) and *GmCAMTA8*-OX (approximately 2.78 to 11.11 %) than the WT (approximately 72.22 to 79.17 %) and Vector-OX (approximately 70.83 to 80.56 %) (Figure 4B). To confirm the drought phenotypes in the *GmCAMTA2*-OX and *GmCAMTA8*-OX plants, we measured the water loss from detached leaves during exposure to drought stress. The water loss of the *GmCAMTA2*-OX and *GmCAMTA8*-OX plants was significantly higher than that of the WT and Vector-OX plants in drought stress responses (Figure 4C). These results suggested that the overexpression of *GmCAMTA2* and *GmCAMTA8* reduced plant tolerance to drought stress.

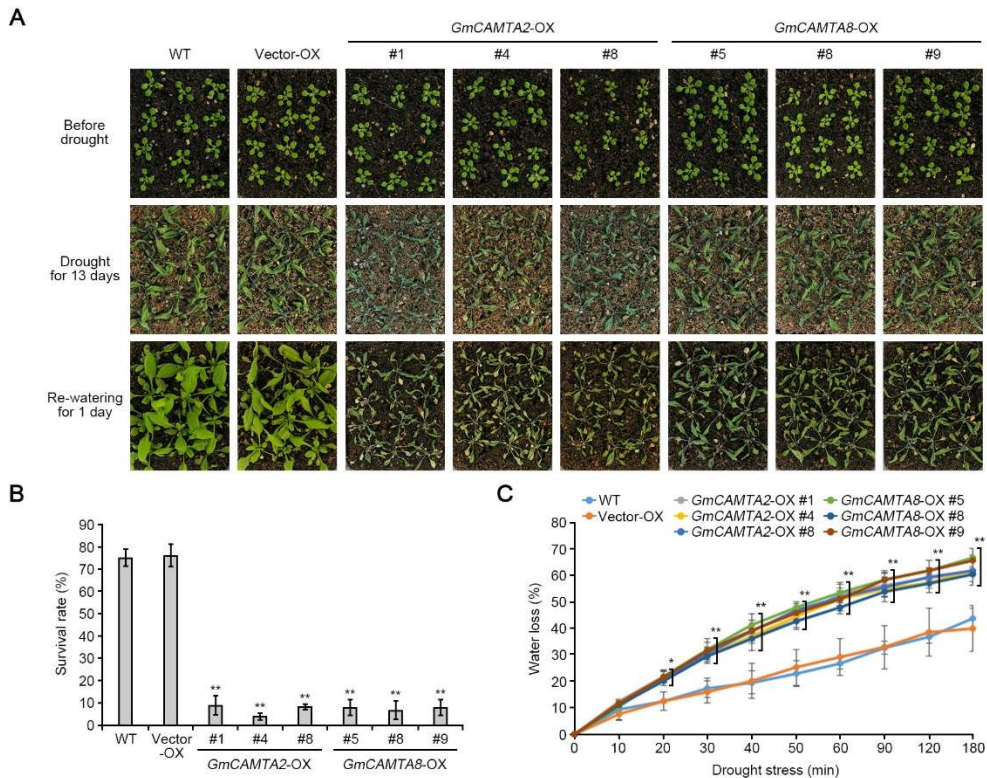


Figure 4. Effect of drought stress on *GmCAMTA2* and *GmCAMTA8* overexpressing transgenic *Arabidopsis*. **(A)** Phenotypes of *GmCAMTA2*-OX and *GmCAMTA8*-OX compared with WT and Vector-OX plants in response to drought stress and re-watering. The plants were grown in soil with sufficient water for two weeks (upper panels), then water was withheld for thirteen days (middle panels). The drought-stressed plants were then re-watered for one day (bottom panels). Each experiment comprised at least 12 plants, and three replicates were performed. **(B)** Comparison of survival rates (%) among *GmCAMTA2*-OX, *GmCAMTA8*-OX, Vector-OX, and WT plants against drought stress. Each experiment comprised at least 12 plants, and error bars represent the SD of three biological replicates. Asterisks indicate significant differences compared with WT plants by Student's t-test (* p -value < 0.05, ** p -value < 0.01). **(C)** Analysis of water loss from detached leaves of three-week-old *GmCAMTA2*-OX, *GmCAMTA8*-OX, Vector-OX, and WT plants under drought stress conditions. Average values of water loss were measured at the indicated time points. Each experiment comprised at least four plants, and error bars represent the SD of three biological replicates. Asterisks indicate significant differences compared with WT plants by Student's t-test (* p -value < 0.05, ** p -value < 0.01).

2.5. Overexpression of *GmCAMTA2* and *GmCAMTA8* regulated the transcripts of stress-responsive genes in drought stress responses

To investigate whether *GmCAMTA2* and *GmCAMTA8* affected the expression patterns of stress-responsive genes during drought stress responses, we performed qRT-PCR analysis of various stress-responsive genes (Figure 5). The expression levels of ABA-induced genes *AtRD29A* and *AtRD29B* were significantly decreased in *GmCAMTA2*-OX (*AtRD29A*; approximately 0.97- to 1.67-fold, *AtRD29B*; approximately 1.18- to 1.69-fold) and *GmCAMTA8*-OX (*AtRD29A*; approximately 1.18- to 1.61-fold, *AtRD29B*; approximately 0.94- to 1.53-fold) compared with those of WT plants (*AtRD29A*; approximately 2.61-fold, *AtRD29B*; approximately 3.60-fold) at the 60 min time point during drought stress. The *Pyrroline-5-Carboxylate Synthase 2* (*P5CS2*) gene was regulated in the biosynthesis of proline, which is an antioxidant in water stress response [39]. The expression of *AtP5CS2* was weakly decreased in *GmCAMTA2*-OX (approximately 0.75- to 0.82-fold) and *GmCAMTA8*-OX (approximately 0.62- to 0.88-fold) compared with that of WT plants (approximately 1.23-fold) at the 60 min time point during drought stress. In addition, the expression of the stress-related marker gene

AtKIN1 was decreased in *GmCAMTA2*-OX (approximately 1.60- to 1.92-fold) and *GmCAMTA8*-OX (approximately 1.37- to 1.50-fold) compared with that of WT plants (approximately 2.76-fold) at the 60 min time point during drought stress. These results suggested that the overexpression of *GmCAMTA2* and *GmCAMTA8* down-regulated the expression of stress-responsive genes during drought stress. Thus, *GmCAMTA2* and *GmCAMTA8* act as negative regulators in drought stress responses.

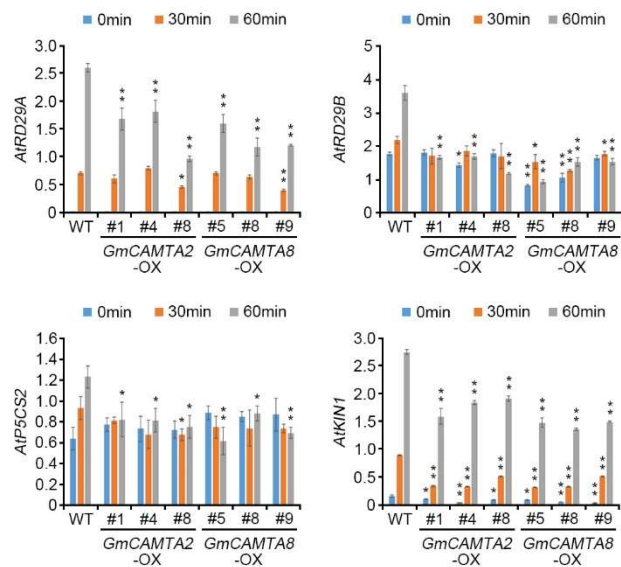


Figure 5. Effect on the transcripts of Arabidopsis stress-responsive genes by the overexpression of *GmCAMTA2* and *GmCAMTA8* in drought stress responses. The expression levels of stress-responsive genes were analyzed using qRT-PCR in *GmCAMTA2*-OX and *GmCAMTA8*-OX plants under drought stress conditions. Total RNA was extracted from 10-day-old *GmCAMTA2*-OX and *GmCAMTA8*-OX *Arabidopsis* at indicated time points to drought stress. The qRT-PCR analysis was performed with each specific primer of the *Arabidopsis* stress-responsive genes, *AtRD29A*, *AtRD29B*, *AtP5CS2*, and *AtKIN1* (Supplementary Table S2). The expression of *Arabidopsis AtTUBULIN2* was used as an internal control for normalization. Error bars represent the SD of two biological replicates, each with three technical replicates. Asterisks indicate significant differences compared with expression levels in WT plants by Student's t-test (* *p*-value < 0.05, ** *p*-value < 0.01).

3. Discussion

The characterization of various *CAMTA* genes, as core transcription factors with CaM binding sites, has been reported in recent years by genome-wide analysis and expression profiles [10,11,16–22,25]. Although 15 *GmCAMTA* genes in soybean have been identified, the functions of *GmCAMTAs* remained almost unknown, except for *GmCAMTA12* [31]. In this study, we identified 15 *GmCAMTA* genes and established that *GmCAMTA* transcripts were controlled by circadian rhythms (Figure 1). Previous studies of functions of *CAMTA* genes were little known about circadian regulation in crops. Plant cellular responses, such as gene expression, development, and metabolic processes, were circadian regulated by circadian oscillators [40,41]. Circadian responses in plants were attributable to cellular oscillations in the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]$) in the surrounding environment, including photoperiod and light intensity [42,43]. The calmodulin-like 24 (CML24), one of the Ca^{2+} -binding proteins, regulated the circadian period in Ca^{2+} -dependent signaling [41]. The most important properties of *CAMTAs* in plants have a high correlation with CaM proteins, as a Ca^{2+} sensor to regulate the gene expression in Ca^{2+} -dependent plant responses [5]. These facts were important to explain that the circadian regulation of *GmCAMTAs* transcripts was mediated by the cellular oscillation of $[\text{Ca}^{2+}]$.

Many *CAMTAs* in crops have been directly or indirectly associated with various developmental and cellular processes, whether through autonomously regulating a major transcription factor or

targeting other transcription factors [5]. The fifteen *GmCAMTAs* in soybean [11], seven *PtCAMTAs* in *Populus trichocarpa* [18], nine *ZmCAMTAs* in maize [21], seven *MtCAMTAs* in *Medicago truncatula* [22], and fifteen *VvCAMTAs* in *Vitis vinifera* [44] were endowed with substantial specificity in the developmental stages by highly tissue-specific expression. Root-specific-induced *PtCAMTA2* and *PtCAMTA3* genes were negatively regulated under cold stress in a short time [18]. In this study, *GmCAMTA2*, *GmCAMTA8*, and *GmCAMTA12* were especially highly expressed in stem tissues compared to other *GmCAMTAs* (Figure 3). The growth of plant tissues, such as flowers, stems, and leaves, were greatly affected by abiotic stress [43]. The specificity in tissues is an important characteristic for investigating the functions of *GmCAMTAs* in diverse molecular processes against environmental stresses. Since plants are constantly exposed to environmental stimuli during their lifetime, they have developed various adaptive mechanisms between the developmental process and stress responses [44,45].

Drought stress is an important restricting factor for crop growth and developmental tissue processes, holding both expansion and elongation down [46]. Since drought stress triggers low turgor pressure in the cell, the osmotic maintenance of cell turgor in crops is of great importance for plant growth and crop survival [46]. In many crops, soybean is highly sensitive to drought stress responses through various morphological changes [47]. When exposed to drought stress, soybean showed momentous changes in most of the tissue morphology, such as a reduction in new branches and trifoliate leaves per plant, growth inhibition of initiation leaves, and reduction in leaf area through repression of lamina expansion [47]. Drought stress has a negative effect on the developmental processes of stems and leaves by decreasing their elongation and expansion [46]. In particular, drought stress often leads to the expression of common morphogenesis- and stress-responsive genes through molecular mechanisms, including reactive oxygen species (ROS)- and phytohormone-dependent signaling [45]. *ATP-dependent metalloprotease 4 (AtFtsH4)* played a tissue-specific role in maintaining the stem cell activity of shoot apical meristem (SAM) stages by ROS accumulation under high-temperature conditions [48]. *MscS-like 2 (MSL2)* and *MSL3* were mediated to cell proliferation by regulation of *WUSCHEL (WUS)* gene expression in osmotic stress responses [49]. The mutation of *NADPH* genes showed unstable shoot development through decreasing the stem cell population [50]. In case of *CAMTA* genes, the relationships between development and stress responses have been studied more in fruit species than in crops. The tomato *SISR2* and *SISR3L* transcripts were affected by ethylene signaling during fruit development and ripening [16]. The expression of peach *PpCAMTA1* was induced in cold stress, but *PpCAMTA3* was repressed with UV-B irradiation during fruit development [32]. Our results indicated that *GmCAMTA2*, *GmCAMTA8*, and *GmCAMTA12* were significantly expressed in both stem tissues and drought stress responses (Figure 2 and 3). When *GmCAMTA2* and *GmCAMTA8* were overexpressed in *Arabidopsis*, *GmCAMTA2-OX* and *GmCAMTA8-OX* displayed the sensitive phenotype under drought stress conditions by losing faster water than the WT plant (Figure 4). These results suggest that *GmCAMTA2* and *GmCAMTA8* act as negative regulators in drought stress responses. Considering the tolerant phenotype produced by *GmCAMTA12* overexpression [31] and sensitive phenotypes by *GmCAMTA2* and *GmCAMTA8* overexpression (Figure 4) under drought stress conditions, *GmCAMTA2*, *GmCAMTA8*, and *GmCAMTA12* may be associated with drought tolerance by different regulating mechanisms. Our results suggest that *GmCAMTA2* and *GmCAMTA8* play an important role in both the development process and drought stress responses by circadian regulation.

4. Materials and Methods

4.1. Plant materials and growth conditions

We used the soybean (*Glycine max* cv. Williams 82) and *Arabidopsis* (*Arabidopsis thaliana* Col-0 ecotype) plants in this study. The soybean plants were cultured in a growth chamber at 28 °C under long-day conditions (16h Light / 8h Dark) and *Arabidopsis* plants in a growth chamber at 22 °C under long-day conditions (16h Light / 8h Dark). To test the expression levels of *GmCAMTAs*, the two-week-old soybean plants were hydroponically cultured in the 1/2 MS medium with circadian rhythms. For

abiotic stress treatments, the two-week-old soybean plants were transferred uniformly in the 1/2 MS medium containing 150mM NaCl (salt stress) and 100 μ M ABA. For cold stress treatment, the two-week-old soybean plants in the 1/2 MS medium were placed on ice (approximately 2 °C). In the treatment of drought stress, the two-week-old soybean or 10-day-old *Arabidopsis* plants after removing the medium were placed in a growth chamber under long-day conditions (16h light / 8h dark). For the expression analysis of *GmCAMTAs* in different tissues, the soybean plants after sowing seeds were grown in the greenhouse under the seasonal cultivation period.

4.2. Identification of *GmCAMTA* genes in soybean

The *GmCAMTA* genes were identified from the soybean genome database at the Phytozome version 13 (<https://phytozome-next.jgi.doe.gov/>). The specific primers for the qRT-PCR and RT-PCR analyses were designed with the IDT (Integrated DNA Technologies) service website (<https://sg.idtdna.com/pages>), based on the sequence information of *GmCAMTAs* (Supplementary Table S2). The full-length coding sequences of *GmCAMTA2* (3,309 bp) and *GmCAMTA8* (2,736 bp) were amplified by PCR using cDNA from soybean plants, and then cloned into the *pMLBart* binary vector under the control of the *CaMV* 35S promoter, which contained a BASTA resistance gene (Supplementary Figure S2). We confirmed the full-length coding sequences through DNA sequencing analysis.

4.3. Generation of *GmCAMTA*-overexpressing *Arabidopsis* transgenic plants

The *pMLBart-GmCAMTA2* and *pMLBart-GmCAMTA8* plasmids were transformed into *Arabidopsis* plants using the *Agrobacterium*-mediated floral-dipping methods. The *GmCAMTA2*- and *GmCAMTA8*-overexpressing transgenic plants were selected on the soil by spraying with 0.2 % BASTA herbicide. The expression levels of *GmCAMTA2* and *GmCAMTA8* in *Arabidopsis* transgenic plants were analyzed using reverse transcription PCR (RT-PCR) with gene-specific primers (Supplementary Table S2).

4.4. Physiological assay of drought stress

To test the physiological phenotypes under drought stress conditions, two-week-old plants grown in soil with sufficient water were not watered for 13 days. After re-watering, the recovery of the drought-treated plants was monitored. The assays for drought sensitivity were thrice-replicated experiments using at least 12 plants for each line in each experiment. To measure the transpirational water loss, leaves from 4-week-old plants in soil were detached and placed on Petri dishes. The fresh weights of detached leaves were measured periodically at the indicated times, and the percentages of water loss were calculated. The water loss assays were replicated three times, using at least four plants for each line in each experiment.

4.5. Analysis of gene expression

The total RNA was isolated from each plant sample using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. To remove any genomic DNA contaminants, purified total RNA was treated with DNaseI (Sigma-Aldrich, St. Louis, MO, USA). For the RT-PCR and quantitative RT-PCR (qRT-PCR) analyses, 1 μ g of total RNA was used for cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA), in accordance with the manufacturer's protocol. The qRT-PCR analysis was performed using the QuantiSpeed SYBR No-ROX Kit (PhileKorea, Seoul, Republic of Korea), and the relative gene expression levels were automatically calculated using the CFX384 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). The expression levels of soybean *GmTUBULIN* and *Arabidopsis AtTUBULIN2* were used as the internal control. The qRT-PCR experiments were performed in three independent replicates. The gene-specific primers used are listed in Supplementary Table S2.

5. Conclusions

In summary, we identified 15 *GmCAMTA* genes in soybean (*Glycine max* cv. Williams 82) plants. A total of 15 *GmCAMTAs* were significantly expressed in accordance with circadian rhythms in the daily clock. The expression levels of most *GmCAMTAs* were induced during various abiotic stresses, including salt, drought, and cold stress, except ABA. Interestingly, *GmCAMTA2*, *GmCAMTA8*, and *GmCAMTA12* were more highly expressed in stem tissues than other *GmCAMTAs*. The overexpression of *GmCAMTA2* and *GmCAMTA8* in *Arabidopsis* suppressed plant tolerance under drought stress conditions. The expression levels of stress-responsive genes were dramatically reduced by overexpressing *GmCAMTA2* and *GmCAMTA8* in drought stress responses. These results suggest that *GmCAMTAs* function in tissue-specific and plant stress responses through rhythmic regulation under drought stress conditions.

Supplementary Materials: Supplementary Figure S1. Diurnal expression of *GmCAMTAs* in the Diurnal web database. Supplementary Figure S2. RT-PCR analysis in *Arabidopsis* transgenic plants overexpressing *GmCAMTA2* and *GmCAMTA8*. Supplementary Table S1. Lists of 15 *GmCAMTA* genes in Phytozome database. Supplementary Table S2. Lists of primers in this study.

Author Contributions: D.B., H.M.C., Y.J.C., H.J.C. and M.C.K. designed and performed the experiments, analyzed data, and wrote the manuscript. B.J.J., S.H.L. and M.S.P. performed some experiments. M.C.K. discussed and commented on the results and revised the manuscript. All authors approved the final manuscript.

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References

- Demidchik, V.; Maathuis, F.J.M. Physiological roles of nonselective cation channels in plants: From salt stress to signalling and development. *New Phytol.* **2007**, *175*, 387–404. <https://doi.org/10.1111/j.1469-8137.2007.02128.x>.
- Demidchik, V.; Shabala, S.; Isayenkov, S.; Cuin, T.A.; Pottosin, I. Calcium transport across plant membranes: Mechanisms and functions. *New Phytol.* **2018**, *220*, 49–69. <https://doi.org/10.1111/nph.15266>.
- Aldon, D.; Mbengue, M.; Mazars, C.; Galaud, J.P. Calcium Signalling in Plant Biotic Interactions. *Int. J. Mol. Sci.* **2018**, *19*, 665. <https://doi.org/10.3390/ijms19030665>.
- Li, Y.; Liu, Y.; Jin, L.; Peng, R. Crosstalk between Ca²⁺ and Other Regulators Assists Plants in Responding to Abiotic Stress. *Plants* **2022**, *11*, 1351. <https://doi.org/10.3390/plants11101351>.
- Iqbal, Z.; Shariq Iqbal, M.; Singh, S.P.; Buaboocha, T. Ca²⁺/Calmodulin Complex Triggers CAMTA Transcriptional Machinery Under Stress in Plants: Signaling Cascade and Molecular Regulation. *Front. Plant Sci.* **2020**, *11*, 598327. <https://doi.org/10.3389/fpls.2020.598327>.
- Kudla, J.; Becker, D.; Grill, E.; Hedrich, R.; Hippler, M.; Kummer, U.; Parniske, M.; Romeis, T.; Schumacher, K. Advances and current challenges in calcium signaling. *New Phytol.* **2018**, *218*, 414–431. <https://doi.org/10.1111/nph.14966>.
- Day, I.S.; Reddy, V.S.; Shad Ali, G.; Reddy, A.S. Analysis of EF-hand-containing proteins in *Arabidopsis*. *Genome Biol.* **2002**, *3*, RESEARCH0056. <https://doi.org/10.1186/gb-2002-3-10-research0056>.
- Sun, Q.P.; Guo, Y.; Sun, Y.; Sun, D.Y.; Wang, X.J. Influx of extracellular Ca²⁺ involved in jasmonic-acid-induced elevation of [Ca²⁺]_{cyt} and *JR1* expression in *Arabidopsis thaliana*. *J. Plant Res.* **2006**, *119*, 343–350. <https://doi.org/10.1007/s10265-006-0279-x>.
- Poovaiah, B.W.; Du, L. Calcium signaling: Decoding mechanism of calcium signatures. *New Phytol.* **2018**, *217*, 1394–1396. <https://doi.org/10.1111/nph.15003>.
- Xiao, P.; Feng, J.W.; Zhu, X.T.; Gao, J. Evolution Analyses of CAMTA Transcription Factor in Plants and Its Enhancing Effect on Cold-tolerance. *Front. Plant Sci.* **2021**, *12*, 758187. <https://doi.org/10.3389/fpls.2021.758187>.
- Wang, G.; Zeng, H.; Hu, X.; Zhu, Y.; Chen, Y.; Shen, C.; Wang, H.; Poovaiah, B.W.; Du, L. Identification and expression analyses of calmodulin-binding transcription activator genes in soybean. *Plant Soil.* **2015**, *386*, 205–221. <https://doi.org/10.1007/s11104-014-2267-6>.

12. Bouché, N.; Scharlat, A.; Snedden, W.; Bouchez, D.; Fromm, H. A novel family of calmodulin-binding transcription activators in multicellular organisms. *J Biol Chem.* **2002**, *277*, 21851–21861. <https://doi.org/10.1074/jbc.M200268200>.
13. Gong, P.; Han, J.; Reddig, K.; Li, H.S. A potential dimerization region of dCAMTA is critical for termination of fly visual response. *J. Biol. Chem.* **2007**, *282*, 21253–21258. <https://doi.org/10.1074/jbc.M701223200>.
14. Finkler, A.; Ashery-Padan, R.; Fromm, H. CAMTAs: Calmodulin-binding transcription activators from plants to human. *FEBS Lett.* **2007**, *581*, 3893–3898. <https://doi.org/10.1016/j.febslet.2007.07.051>.
15. Rhoads, A.R.; Friedberg, F. Sequence motifs for calmodulin recognition. *FASEB J.* **1997**, *11*, 331–340. <https://doi.org/10.1096/fasebj.11.5.9141499>.
16. Yang, T.; Peng, H.; Whitaker, B.D.; Conway, W.S. Characterization of a calcium/calmodulin-regulated SR/CAMTA gene family during tomato fruit development and ripening. *BMC Plant Biol.* **2012**, *12*, 19. <https://doi.org/10.1186/1471-2229-12-19>.
17. Zhou, Q.; Zhao, M.; Xing, F.; Mao, G.; Wang, Y.; Dai, Y.; Niu, M.; Yuan, H. Identification and Expression Analysis of CAMTA Genes in Tea Plant Reveal Their Complex Regulatory Role in Stress Responses. *Front Plant Sci.* **2022**, *13*, 910768. <https://doi.org/10.3389/fpls.2022.910768>.
18. Wei, M.; Xu, X.; Li, C. Identification and expression of CAMTA genes in *Populus trichocarpa* under biotic and abiotic stress. *Sci. Rep.* **2017**, *7*, 17910. <https://doi.org/10.1038/s41598-017-18219-8>.
19. Meer, L.; Mumtaz, S.; Labbo, A.M.; Khan, M.J.; Sadiq, I. Genome-wide identification and expression analysis of calmodulin-binding transcription activator genes in banana under drought stress. *Sci. Hortic.* **2019**, *244*, 10–14. <https://doi.org/10.1016/j.scienta.2018.09.022>.
20. Saeidi, K.; Zare, N.; Baghizadeh, A.; Asghari-Zakaria, R. *Phaseolus vulgaris* genome possesses CAMTA genes, and *phavuCAMTA1* contributes to the drought tolerance. *J Genet.* **2019**, *98*, 31. <https://doi.org/10.1007/s12041-019-1069-2>.
21. Yue, R.; Lu, C.; Sun, T.; Peng, T.; Han, X.; Qi, J.; Yan, S.; Tie, S. Identification and expression profiling analysis of calmodulin-binding transcription activator genes in maize (*Zea mays* L.) under abiotic and biotic stresses. *Front. Plant Sci.* **2015**, *6*, 576. <https://doi.org/10.3389/fpls.2015.00576>.
22. Yang, Y.; Sun, T.; Xu, L.; Pi, E.; Wang, S.; Wang, H.; Shen, C. Genome-wide identification of CAMTA gene family members in *Medicago truncatula* and their expression during root nodule symbiosis and hormone treatments. *Front. Plant Sci.* **2015**, *6*, 459. <https://doi.org/10.3389/fpls.2015.00459>.
23. Pandey, N.; Ranjan, A.; Pant, P.; Tripathi, R.K.; Ateek, F.; Pandey, H.P.; Patre, U.V.; Sawant, S.V. CAMTA 1 regulates drought responses in *Arabidopsis thaliana*. *BMC Genom.* **2013**, *14*, 216. <https://doi.org/10.1186/1471-2164-14-216>.
24. Doherty, C.J.; Van Buskirk, H.A.; Myers, S.J.; Thomashow, M.F. Roles for Arabidopsis CAMTA transcription factors in cold-regulated gene expression and freezing tolerance. *Plant Cell.* **2009**, *21*, 972–984. <https://doi.org/10.1105/tpc.108.063958>.
25. Noman, M.; Aysha, J.; Keteouli, T.; Yang, J.; Du, L.; Wang, F.; Li, H. Calmodulin binding transcription activators: An interplay between calcium signalling and plant stress tolerance. *J Plant Physiol.* **2021**, *256*, 153327. <https://doi.org/10.1016/j.jplph.2020.153327>.
26. Du, L.; Ali, G.S.; Simons, K.A.; Hou, J.; Yang, T.; Reddy, A.S.; Poovaiah, B.W. Ca²⁺/calmodulin regulates salicylic-acid-mediated plant immunity. *Nature* **2009**, *457*, 1154–1158. <https://doi.org/10.1038/nature07612>.
27. Galon, Y.; Nave, R.; Boyce, J.M.; Nachmias, D.; Knight, M.R.; Fromm, H. Calmodulin-binding transcription activator (CAMTA) 3 mediates biotic defense responses in *Arabidopsis*. *FEBS Lett.* **2008**, *582*, 943–948. <https://doi.org/10.1016/j.febslet.2008.02.037>.
28. Mitsuda, N.; Isono, T.; Sato, M.H. *Arabidopsis* CAMTA family proteins enhance V-PPase expression in pollen. *Plant Cell Physiol.* **2003**, *44*, 975–981. <https://doi.org/10.1093/pcp/pcg137>.
29. Shkolnik, D.; Finkler, A.; Pasmanik-Chor, M.; Fromm, H. CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR 6: A Key Regulator of Na⁺ Homeostasis during Germination. *Plant Physiol.* **2019**, *180*, 1101–1118. <https://doi.org/10.1104/pp.19.00119>.
30. Koo, S.C.; Choi, M.S.; Chun, H.J.; Shin, D.B.; Park, B.S.; Kim, Y.H.; Park, H.M.; Seo, H.S.; Song, J.T.; Kang, K.Y.; Yun, D.J.; Chung, W.S.; Cho, M.J.; Kim, M.C. The calmodulin-binding transcription factor OsCBT suppresses defense responses to pathogens in rice. *Mol. Cells.* **2009**, *27*, 563–570. <https://doi.org/10.1007/s10059-009-0081-4>.

31. Noman, M.; Jameel, A.; Qiang, W.D.; Ahmad, N.; Liu, W.C.; Wang, F.W.; Li, H.Y. Overexpression of *GmCAMTA12* Enhanced Drought Tolerance in Arabidopsis and Soybean. *Int. J. Mol. Sci.* **2019**, *20*, 4849. <https://doi.org/10.3390/ijms20194849>.
32. Yang, C.; Li, Z.; Cao, X.; Duan, W.; Wei, C.; Zhang, C.; Jiang, D.; Li, M.; Chen, K.; Qiao, Y.; Liu, H.; Zhang, B. Genome-Wide Analysis of Calmodulin Binding Transcription Activator (CAMTA) Gene Family in Peach (*Prunus persica* L. Batsch) and Ectopic Expression of *PpCAMTA1* in Arabidopsis *camta2,3* Mutant Restore Plant Development. *Int. J. Mol. Sci.* **2022**, *23*, 10500. <https://doi.org/10.3390/ijms231810500>.
33. Tripathi, N.; Khare, D. Molecular approaches for genetic improvement of seed quality and characterization of genetic diversity in soybean: A critical review. *Biotechnol. Lett.* **2016**, *38*, 1645–1654. <https://doi.org/10.1007/s10529-016-2154-8>.
34. Haun, W.J.; Hyten, D.L.; Xu, W.W.; Gerhardt, D.J.; Albert, T.J.; Richmond, T.; Jeddeloh, J.A.; Jia, G.; Springer, N.M.; Vance, C.P.; Stupar, R.M. The composition and origins of genomic variation among individuals of the soybean reference cultivar Williams 82. *Plant Physiol.* **2011**, *155*, 645–655. <https://doi.org/10.1104/pp.110.166736>.
35. Xu, X.; Yuan, L.; Xie, Q. The circadian clock ticks in plant stress responses. *Stress Biol.* **2022**, *2*, 15. <https://doi.org/10.1007/s44154-022-00040-7>.
36. Ma, X.; Su, Z.; Ma, H. Molecular genetic analyses of abiotic stress responses during plant reproductive development. *J. Exp. Bot.* **2020**, *71*, 2870–2885. <https://doi.org/10.1093/jxb/eraa089>.
37. Sabbioni, G.; Funck, D.; Forlani, G. Enzymology and Regulation of δ 1-Pyrroline-5-Carboxylate Synthetase 2 From Rice. *Front. Plant Sci.* **2021**, *12*, 672702. <https://doi.org/10.3389/fpls.2021.672702>.
38. Zhou, L.; Zhou, J.; Xiong, Y.; Liu, C.; Wang, J.; Wang, G.; Cai, Y. Overexpression of a maize plasma membrane intrinsic protein ZmPIP1;1 confers drought and salt tolerance in *Arabidopsis*. *PLoS ONE* **2018**, *13*, e0198639. <https://doi.org/10.1371/journal.pone.0198639>.
39. Más, P. Circadian clock function in *Arabidopsis thaliana*: Time beyond transcription. *Trends Cell Biol.* **2008**, *18*, 273–281. <https://doi.org/10.1016/j.tcb.2008.03.005>.
40. Martí Ruiz, M.C.; Hubbard, K.E.; Gardner, M.J.; Jung, H.J.; Aubry, S.; Hotta, C.T.; Mohd-Noh, N.I.; Robertson, F.C.; Hearn, T.J.; Tsai, Y.C.; Dodd, A.N.; Hannah, M.; Carré, I.A.; Davies, J.M.; Braam, J.; Webb, A.A.R. Circadian oscillations of cytosolic free calcium regulate the *Arabidopsis* circadian clock. *Nat. Plants* **2018**, *4*, 690–698. <https://doi.org/10.1038/s41477-018-0224-8>.
41. Love, J.; Dodd, A.N.; Webb, A.A. Circadian and diurnal calcium oscillations encode photoperiodic information in *Arabidopsis*. *Plant Cell* **2004**, *16*, 956–966. <https://doi.org/10.1105/tpc.020214>.
42. Tataroglu, O.; Zhao, X.; Busza, A.; Ling, J.; O'Neill, J.S.; Emery, P. Calcium and SOL Protease Mediate Temperature Resetting of Circadian Clocks. *Cell* **2015**, *163*, 1214–1224. <https://doi.org/10.1016/j.cell.2017.09.013>.
43. Zhao, L.; Li, Y.; Xie, Q.; Wu, Y. Loss of CDKC2 increases both cell division and drought tolerance in *Arabidopsis thaliana*. *Plant J.* **2017**, *91*, 816–828. <https://doi.org/10.1111/tpj.13609>.
44. Shangguan, L.; Wang, X.; Leng, X.; Liu, D.; Ren, G.; Tao, R.; Zhang, C.; Fang, J. Identification and bioinformatic analysis of signal responsive/calmodulin-binding transcription activators gene models in *Vitis vinifera*. *Mol. Biol. Rep.* **2014**, *41*, 2937–2949. <https://doi.org/10.1007/s11033-014-3150-5>.
45. Zhu, J.K. Abiotic Stress Signaling and Responses in Plants. *Cell* **2016**, *167*, 313–324. <https://doi.org/10.1016/j.cell.2016.08.029>.
46. Jaleel, C.A.; Manivannan, P.; Wahid, A.; Farooq, M.; Al-Juburi, H.J.; Somasundaram, R.; Panneerselvam, R. Drought stress in plants: A review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.* **2009**, *11*, 100–105. doi: 08–305/IGC-DYT/2009/11–1–100–105.
47. Mangena, P. Water stress: Morphological and anatomic changes in soybean (*Glycine max* L.) plants. In *Plant, Abiotic Stress and Responses to Climate Change*, ed V. Andjelkovic (London: Intech Open); 2018; pp. 9–31. <https://doi.org/10.5772/intechopen.72899>.
48. Lee, H. Stem Cell Maintenance and Abiotic Stress Response in Shoot Apical Meristem for Developmental Plasticity. *J. Plant Biol.* **2018**, *61*, 358–365. <https://doi.org/10.1007/s12374-018-0301-6>.

49. Dolzblasz, A.; Smakowska, E.; Gola, E.M.; Sokołowska, K.; Kicia, M.; Janska, H. The mitochondrial protease AtFTSH4 safeguards *Arabidopsis* shoot apical meristem function. *Sci. Rep.* **2016**, *6*, 28315. <https://doi.org/10.1038/srep28315>.
50. Wilson, M.E.; Mixdorf, M.; Berg, R.H.; Haswell, E.S. Plastid osmotic stress influences cell differentiation at the plant shoot apex. *Development* **2016**, *143*, 3382–3393. <https://doi.org/10.1242/dev.136234>.