Identification, Expression, and Functional Characterization of ScCaM in Response to Various Stresses in Sugarcane

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Abstract: Calmodulin (CaM), as an important factor in the calcium signaling pathway, is widely involved in plant growth and development regulation and responses to external stimuli. In this study, the full-length sequence of the ScCaM gene (GenBank: GQ246454) was isolated from the leaves of a Saccharum spp. hybrid. Prokaryotic expression showed that ScCaM could be solubly expressed and purified in Escherichia coli BL21. Subcellular localization confirmed that ScCaM was localized in the plasma membrane and nucleus of cells. A quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis revealed that ScCaM can be induced by various stresses, including sodium chloride (NaCl), chromium trichloride (CrCl3), salicylic acid (SA), and methyl jasmonate (MeA). Ectopic expression in Arabidopsis thaliana demonstrated that ScCaM can affect the growth and development of transgenic plants. Moreover, the qRT-PCR analysis indicated that the overexpression of the allogenic ScCaM gene inhibits the expression of AtSTM, leading to the phenomenon of multiple-tillering in transgenic A. thaliana. Furthermore, the expression patterns of ScCaM under abiotic stress and phytohormone stimulation in transgenic A. thaliana confirmed that ScCaM was involved in the responses to phytohormone, high salt, and heavy metal stresses. The present study provided valuable information and facilitates further investigation into the function of ScCaM in the future.

Keywords: sugarcane; calmodulin; prokaryotic expression; subcellular localization; stress; transient overexpression

1. Introduction

As the most important Ca\(^{2+}\) receptor, calmodulins (CaMs), which belong to a vital class of Ca\(^{2+}\) binding proteins, are widely present in eukaryotes [1]. Previous studies have shown that CaM, which usually comprises 148 amino acid residues and four EF-hands, was highly conserved in evolution [2]. Commonly, CaM consists of two domains (a globular N-terminal domain and a highly homologous C-terminal domain) with two E–F hands, respectively [3]. It is noteworthy that both domains of CaM adopt different conformations in the absence or presence of Ca\(^{2+}\) [3].

In plants, CaM genes have been identified from different plants, including Arabidopsis thaliana, Oryza sativa, Sorghum bicolor, and so on [4]. For example, there are a total of seven highly similar calmodulins with only a few amino acid differences in A. thaliana [5]. There are five and eight CaM genes in O. sativa and S. bicolor, respectively [4]. Previous studies have shown that, by binding with Ca\(^{2+}\), CaMs can interact with the downstream...
calmodulin-binding protein (CaMBP) to activate these enzymes to play important roles in the ion transport, gene regulation, cytoskeleton construction, disease resistance, and stress tolerance of plants [6]. For instance, CaM can repress the expression of EDS1 through binding and activating CAMTA3, which results in decreased salicylic acid levels and disease resistance [7]. The binding with Ca\(^{2+}\)/calmodulin is important for the function of DWARF1 (DWF1) in plant development [8]. Additionally, overexpression of GmCaM4 can promote tolerance to salt stress and resistance to pathogens in soybean [9]. AtCaM was found to function at the growth of photomorphogenesis [10]. Liu et al. found that TaCaM5 is involved in the regulation of wheat under injury, high salt, low temperature, and drought stress [11].

Sugarcane (Saccharum spp. complex), an important sugar-yielding and energy crop, has important biological and economic value [12]. However, the development of the sugar industry has been restricted by many different environmental stresses [13]. CaMs, as vital Ca\(^{2+}\) sensors, have multiple functions in plants, such as regulating growth and development and enhancing resistance to stresses [14,15]. In this study, one ScCaM gene was isolated from sugarcane, and its sequence was bioinformatically analyzed. Then, a quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to detect the expression profiles under various stresses, such as sodium chloride (NaCl), chromium trichloride (CrCl\(_3\)), salicylic acid (SA), and methyl jasmonate (MeJA). Furthermore, the prokaryotic expression vector of ScCaM was constructed and its expression was induced in Escherichia coli. Moreover, the subcellular localization of ScCaM was analyzed. Finally, the overexpression of ScCaM in A. thaliana was conducted to reveal the phenotype and expression patterns under different stresses. The present study herein may facilitate further investigation into the clear functions of CaM genes in sugarcane.

2. Materials and Methods

2.1. Plant Materials

The Saccharum spp. hybrid (sugarcane cultivar ROC22) was provided by the Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture (Fuzhou, China). The sugarcane stems were planted in high-temperature sterilized sand. When the seedlings grew to six leaves, we selected the seedlings with similar growth states and transferred them to a hydroponic culture for two days. Then, we transferred them to 1/10 Hongland nutrient solution for culturing for seven days. For SA and MeJA treatment, 5 mM of SA and 100 µM of MeJA, respectively, were sprayed on the leaves. For NaCl and CrCl\(_3\) treatment, 250 mM of NaCl, and 50 µM of CrCl\(_3\), respectively, were used to soak the seedlings. Three biological replicates for each group were prepared. The leaves for hormone treatments and the whole seedlings for NaCl treatment were collected at 0 h, 3 h, 12 h, and 24 h, and frozen in liquid nitrogen quickly, then stored at −80 °C until total RNA extraction.

2.2. Gene Isolation and Bioinformatics Analysis

Based on our previous full-length cDNA library of sugarcane leaves, the gene of ScCaM was obtained (Saccharum spp. hybrid (ROC22) [16]. ExPASy (http://web.expasy.org/protparam/, accessed on 25 March 2019), TMHMM v2.0 program (http://www.cbs.dtu.dk/services/TMHMM/, accessed on 25 March 2019), and SignalP (http://www.cbs.dtu.dk/services/SignalP/, accessed on 25 March 2019) were applied to predict the primary protein structures, transmembrane domains, and signal peptides, respectively. SMART (http://smart.embl.de/, accessed on 25 March 2019), SOMPA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html/, accessed on 25 March 2019), and SWISS-MODEL (https://swissmodel.expasy.org/, accessed on 25 March 2019) were used to detect the conserved domains, secondary structure, and three-dimensional structure of ScCaM, respectively. MEGA X was applied to infer the phylogenetic tree using the NJ method.
2.3. Prokaryotic Expression in E. coli BL21 (DE3) Cells

The ORF of ScCaM was amplified by using the ScCaM primers (Table S1). The PCR product and the pGEX-4T vector, both digested with BamHI and EcoRI, were ligated. The recombinant plasmid (pGEX-4T-ScCaM) and empty plasmid (pGEX-4T) were transformed into E. coli BL21 (DE3) competent cells, respectively. In order to study the expression condition of the GST-ScCaM protein, different inducing temperatures (16°C, 28°C, and 37°C), time periods (0 h, 2 h, 8 h, and 20 h), and isopropyl β-D-thiogalactoside (IPTG) concentrations (0.1 mM, 0.5 mM, and 1.0 mM) were used. Finally, the induced protein was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2.4. Subcellular Localization Analysis

The open reading frame (ORF) of ScCaM without a stop codon was amplified by the primers ScCaM-pBWA(V)HS (Table S1) to insert into the Aar plasmid restriction site of the pBWA(V)HS-osgfp vector. Then, the recombinant vector pBWA(V)HS-ScCaM-osgfp was transformed into the competent cells of Agrobacterium tumefaciens, strain GV3101. The recombinant vector pBWA(V)HS-ScCaM-GLoosgfp and the nucleus marker vector (NLS-mCherry), or the cell membrane marker vector (OsMCA1-mCherry), were co-transformed in Nicotiana benthamiana leaves. Agrobacterium-mediated transient expression in N. benthamiana leaves was performed according to Su et al. [17]. The subcellular localization of the fusion protein was visualized by Olympus FV1000 [18].

2.5. qRT-PCR Analysis

The qRT-PCR primers of ScCaM were designed by primer software (version 5.0, San Francisco, USA). The reaction procedure was as follows: 95°C for 2 min, 40 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 2 min. The melting curves were analyzed after 40 cycles. Each qRT-PCR contains three biological replicates and two technical replicates. The relative expression level of qRT-PCR data was calculated by the $2^{-\Delta\Delta C_t}$ method [19]. The primers used in the qRT-PCR analysis are listed in Table S1. Data were expressed as the mean ± standard error (SE), and significance ($p < 0.05$) was calculated using a one-way ANOVA followed by Duncan’s new multiple range test.

2.6. Phenotype and Expression Pattern Analysis of Arabidopsis thaliana Overexpressing ScCaM Gene under Different Exogenous Stresses

The overexpression vector pBWA(V)HS-35S-ScCAM was constructed by the in-fusion cloning technique and then transformed into Agrobacterium strain GV1301 competent cells. For transformation into A. thaliana (Col-0), wild-type A. thaliana flowers were dropped and infiltrated into Agrobacterium strain GV1301 competent cells containing recombinant plasmid pBWA(V)HS-35S-ScCAM. Using homogenin and RT-PCR amplification of the HYG gene, the T3 generation of A. thaliana transgenic lines was selected. The A. thaliana transgenic lines in the T3 generation were planted in 1/2 Murashige and Skoog (MS) medium. When A. thaliana had cultured to 7–8 true leaves, the expression of ScCAM in all T3 generation lines was analyzed by qRT-PCR, and the lines with higher expression levels were selected for subsequent experiments. The whole seedlings were collected and frozen in liquid nitrogen quickly, then stored at $-80\degree$C until total RNA extraction.

To further clarify the function of the ScCaM gene, the expression patterns of the ScCaM gene in transgenic A. thaliana under different exogenous stresses were analyzed by qRT-PCR. The T3 seeds with the highest ScCaM gene expression were selected and planted in 1/2 MS medium. When A. thaliana had cultured to 7–8 true leaves, the plants were immersed in 5 mM of SA, 100 μM of MeJA, 100 mM of NaCl, and 50 mM of CrCl$_3$ in 1/2 MS liquid medium for stress treatment. The samples were harvested at 0 h, 1h, 3 h, 6 h, 12 h, and 24 h for each treatment, frozen in liquid nitrogen, and stored at $-80\degree$C until total RNA extraction. All primers used in the qRT-PCR are listed in Table S1.
3. Results

3.1. Identification and Sequence Features of ScCaM

In this study, the full-length cDNA sequence of ScCaM (GenBank Acc No. GQ246454) was isolated. It was 925 bp long, with a 450-bp ORF that encoded a polypeptide of 149 amino acids (Figure 1A). The physical and chemical properties of ScCaM showed that its molecular mass, theoretical pI, instability index (II), and grand average of hydropathicity (GRAVY) were 16.83 kDa, 4.11, −0.602, and 23.23, respectively. These results indicate that ScCaM is an acidic, stable, hydrophilic protein. The prediction results of TMHMM and SignalP 5.0 Sever demonstrated that ScCaM has no transmembrane region and no signal peptide, so it is not a secretory protein. According to SMART, the amino acid sequence of the ScCaM has four typical EF-hand conservative structure domains (12–40, 48–76, 85–113, and 121–149 amino acids in length) (Figure 1B). The results of the protein secondary structures show that ScCaM mainly consists of 92 α-helices (65.71%), 36 random coils (24.16%), 12 β-sheets (8.05%), and 9 extension chains (6.04%). The three-dimensional model of ScCaM demonstrates that the protein has two EF-hands at the C-terminal and N-terminal, respectively (Figure 1C).

Figure 1. The analysis of sequence, conserved domains, and protein structure. (A) Complete cDNA and deduced amino acid sequences of the ScCaM gene. (B) The conserved domains of ScCaM. (C) The three-dimensional model of ScCaM.
3.2. Sequence and Phylogenetic Analysis of CaM Proteins

According to the results of blastp in NCBI, the ScCaM from sugarcane shares high sequence similarity with CaM homologs from ten other plant species, ranging from 98% to 99% similarity. Multiple sequence alignments of ScCaM with 11 CaM proteins from other species by ClustalX showed that the CaMs were highly conserved (Figure 2A). All CaMs contained four EF-hands, which consist of an E-helix, a loop, and an F-helix. It is worth noting that the E-helix starts with a glutamic acid (E), and hydrophobic amino acids are distributed in sequence according to the rule of “h*h*h”, where “h” represents hydrophobic amino acids and “*” represents any amino acid. This special distribution pattern also applies to the F-helix of the first and third EF-hand, while the F-helix distribution patterns of the second and fourth EF-hands are slightly different from this pattern. About the loop, the first (site 1) and third (site 3) amino acids are both the much-conserved aspartic acid (D). The fifth (site 5) amino acid is usually aspartic acid (D) or asparagine (N), but the seventh (site 7) and ninth (site 9) amino acids are not conserved. The 12th (site 0) is a very conserved glutamic acid (E) with the function of linking two coordination sites. The sixth (site 6) amino acid, glycine (G), functions by maintaining the structure of the ring, while the isoleucine (I) at site 8 plays a part in forming hydrogen bonds to pair with other chiral molecular rings. The phylogenetic analysis indicates that these CaM proteins could be divided into two groups of dicots (Group A) and monocots (Group B) (Figure 2B). Moreover, ScCaM has a close relationship with CaMs from Zea mays and Triticum aestivum.

3.3. Expression of ScCaM in E. coli BL21 (DE3) Strain

In order to explore the prokaryotic expression conditions of the GST-ScCaM fusion protein, the expression temperature, duration, and IPTG concentration were compared and analyzed. As illustrated in Figure 3A, the GST-ScCaM fusion protein was expressed most clearly after 0.5 mM of IPTG induction for 20 h at 16 °C (Figure 3A). According to the GST fusion protein purification method, the cells were lysed, and the proteins in the supernatant were extracted and purified by affinity column chromatography. As EXPASY Protparam predicted, the molecular weight of the ScCaM protein was 16.83 kDa, while that of GST protein was 26 kDa. As Figure 3B demonstrates, the target protein of GST-ScCaM, which weighs about 43 kDa, was successfully induced and purified.

3.4. Subcellular Localization of ScCaM in N. benthamiana Cells

To confirm the subcellular localization of ScCaM, the leaves of tobacco were injected with pBWA(V)HS-ScCaM-GFP and OsMCA1-mcherry, and with pBWA(V)HS-ScCaM- GFP and NLS-mcherry. Previous studies have shown that OsMCA1-mcherry [20] is mainly expressed in the cell membrane, while NLS-mcherry [21] is expressed in the nucleus. Here, our results show that the ScCaM proteins were mainly located in the plasma membrane and nucleus (Figure 4).

3.5. Gene Expression Patterns of ScCaM under Abiotic Stress and Phytohormone Stimulation in Sugarcane

A qRT-PCR was used to analyze the expression levels of ScCaM in sugarcane under different exogenous stresses (Figure 5). In general, compared with the expression level at 0 h, ScCaM was induced at 3 h. Under NaCl stress, the expression level of ScCaM was induced at 3 h and 24 h. In the CrCl$_3$-treated samples, ScCaM had the highest expression at 3 h, with the expression level being 7.1 times that of the control. However, the expression level of ScCaM was inhibited at 12 h and 24 h. As for SA treatment, the expression level of ScCaM was up-regulated at 3 h and 24 h and peaked at 24 h. Under MeJA treatment, the expression level of ScCaM was up-regulated at all treatment time points and peaked at 24 h at about 27 times that of the control.
Figure 2. The sequence homology and phylogenetic analysis. (A). Sequence homology analysis of ScCaM and the other 11 CaMs from other plant species. The black, blue, and yellow rectangles represent the E-helix, loop, and F-helix, respectively. “h” represents hydrophobic amino acids and “*” represents any amino acid. (B). Phylogenetic analysis of CaM proteins from 12 plant species. All the corresponding GenBank accessions are listed in Table S2.
3.4. Subcellular Localization of ScCaM in N. benthamiana Cells

Prokaryotic expression of the pGEX-4T-ScCaM fusion protein in Escherichia coli BL21 (DE3). (A). Prokaryotic expression of the pGEX-4T-ScCaM fusion protein in Escherichia coli BL21 (DE3) by 0.5 mM of IPTG induced at 16 °C. Note: M, protein marker; 0, control; 1–4, pGEX-4T-ScCaM induction for 0, 2, 8, and 20 h, respectively. (B). Prokaryotic expression of the pGEX-4T-ScCaM fusion protein in Escherichia coli BL21 (DE3) after 0.5 mM of IPTG induced for 20 h at 16 °C. Note: M, protein marker; 1, pGEX-4T-ScCaM induction for 20 h.

Figure 4. Subcellular localization analysis of ScCaM in Nicotiana benthamiana leaves. (A). The subcellular localization analysis of pBWA(V)HS-GFP. (B). The subcellular localization analysis of pBWA(V)HS-ScCaM-GFP and OsMCA1-mCherry. (C). The subcellular localization analysis of pBWA(V)HS-ScCaM-GFP and NLS-mCherry.

3.6. Overexpression of ScCaM Affect Growth and Development in A. thaliana

Through homomycin and RT-PCR amplification of the HYG gene, five homozygous transgenic A. thaliana lines of the T3 generation were selected. A qRT-PCR showed that the expression levels of ScCaM in the five lines were different (Figure 6D), and accordingly, the T3-1, T3-4, and T3-5 lines, which had higher expression levels, were retained as overexpressed materials for subsequent studies.
transgenic A. thaliana showed multiple tillers but fewer fruit pods, indicating that this transgenic line may conduct the calcium receptor signal on the plasma membrane in the medial part of the plasma membrane. If ScCaM is located in the nucleus, we can speculate that ScCaM may conduct the calcium receptor signal on the plasma membrane in the medial part of the plasma membrane.

Figure 5. Expression profile of the ScCaM gene under different stresses in sugarcane. GAPDH was used for the normalization of the transcript levels. Asterisks indicate a significant difference, as determined by a one-way ANOVA (treatment: control/salt) between subjects, followed by Duncan’s new multiple range test (ns indicates no significant difference, * p < 0.05, ** p < 0.01). The F-statistics, degrees of freedom, and p-value for each factor or interaction were shown in Table S3.

Figure 6. Overexpression of ScCaM in Arabidopsis thaliana. (A). Phenotypes of 3-week-old plants of the transgenic line (T3-5) and the wild type (Col-0). (B). Phenotypes of 6-week-old plants of the transgenic line (T3-5) and the wild type (Col-0). (C). Phenotypes of 9-week-old plants of the transgenic line (T3-5) and the wild type (Col-0). (D). The transcripts of ScCaM in the transgenic Arabidopsis thaliana. (E). The transcripts of AtSTM in the Arabidopsis thaliana of the transgenic line (T3-5) and the wild type (Col-0). AtACT2 was used for the normalization of the transcript levels. Asterisks indicate a significant difference, as determined by a one-way ANOVA (treatment: WT/transgenic), followed by Duncan’s new multiple range test (ns indicates no significant difference, ** p < 0.01). The F-statistics, degrees of freedom, and p-value for each factor or interaction are shown in Tables S4 and S5.
The phenotypic characteristics of transgenic and wild-type A. thaliana demonstrated that there was no significant difference between transgenic and wild-type A. thaliana at the early growth stage. After three weeks of growth, the transgenic A. thaliana showed slower growth and more premature senescence of old leaves compared with the wild-type A. thaliana (Figure 6A). In the reproductive growth stage, the stem growth of transgenic A. thaliana was slower than that of wild-type A. thaliana, and the spacing between fruit pods was shorter (Figure 6B). At the maturation stage of the fruit pods, the transgenic A. thaliana showed multiple tillers but fewer fruit pods, indicating a significant decrease in seed number compared with the wild-type A. thaliana (Figure 6C).

Based on these above results, a qRT-PCR was further used to detect the expression of AtMAX1, AtBRC1, AtCLV, AtWUS, and AtSTM, which relate to the growth and development in transgenic and wild-type A. thaliana (Figure 6E). It is worth noting that the expression of AtSTM in transgenic A. thaliana was lower than that of the wild-type A. thaliana, but no significant difference was observed in the expression of other genes (AtMAX1, AtBRC1, AtCLV, and AtWUS) (Figures 6E and S1).

4. Discussion

Calcium (Ca$^{2+}$), as an important second messenger, plays a vital role in signal transduction [22]. Calmodulin (CaM) is one of the most crucial calcium sensor proteins of signal transduction [23]. In the present study, one CaM gene was identified from a Saccharum spp. hybrid (ROC22). In addition, the ScCaM protein was isolated and its subcellular localization was analyzed. A qRT-PCR was used to verify the gene expression patterns of ScCaM under abiotic stress and phytohormone stimulation in sugarcane. Furthermore, the phenotypic characteristics and gene expression patterns were analyzed in transgenic A. thaliana. In summary, this study may provide important clues and a foundation for the further investigation of the function of the ScCaM gene in sugarcane.

Determining the subcellular localization of proteins in plant cells is important to provide useful clues for studying their functions [24]. In this study, the ScCaM protein was found to be located in the plasma membrane and nucleus, which was consistent with the locations of CaM proteins in Pisum sativum [25] and Cucumis sativus [26]. As ScCaM was located in the plasma membrane, we suppose that ScCAM, as a typical calmodulin, may conduct the calcium receptor signal on the plasma membrane in the medial part of the plasma membrane. If ScCaM is located in the nucleus, we can speculate that ScCaM may interact with transcription factors such as AtCaM7 [10] and GmCaM4 [9] to play an important role as a transcriptional regulator. Previous studies have shown that the calcium-dependent calmodulin-binding transcription factor CAMAT3 needs to bind CaM to activate downstream gene expression in the salicylic acid pathway [27]. Our results indicate that ScCaM may also play a role as a ligand of calmodulin-binding transcription factors that activate downstream pathways of the CAMTA proteins.

Previous studies have reported that CaM genes play important roles under various environmental stimuli [28,29]. In this study, the ScCaM gene was found to not only respond to stresses (NaCl and CrCl$_3$), but also to phytohormone (SA and MeJA) stimulation. It is worth noting that ScCaM was usually induced at 3 h and 24 h under different stimuli. These results imply that ScCaM might not only be involved in the early response of calcium ion signals on the cell surface but also in the transcriptional activation of downstream genes.

In order to further verify the function of the ScCaM gene, ScCaM was heterologously overexpressed in A. thaliana. It is interesting that the homozygous transgenic lines began to show slower leaf growth and more premature senescence phenotypes after growing to three weeks of age under long light conditions. In addition, it was found that the internode of the stem became shorter and more tillered, which led to a decrease in fruit pod number and seed number. The results suggest that the overexpression of ScCaM may affect the lateral bud development and later reproductive development of A. thaliana. It is known that leaf senescence is the terminal stage of leaf development, including the yellowing of leaves caused by the degradation of chlorophyll and the reduction in photosynthesis.
and protein synthesis [30]. Ritu Kushwaha et al. have found that the overexpression of CAM7 can regulate the growth of *A. thaliana* [10]. In addition, the *Calmodulin 1* (*CaM1*) gene, which encodes the Ca\(^{2+}\)-binding protein calmodulin 1, positively regulates ROS production, leaf senescence, and ABA response in *Arabidopsis* [30]. Similarly, the overexpression of *ScCaM* had an impact on the growth and development of *A. thaliana*. By detecting the expression of several genes related to growth and development, *AtSTM* was found to be influenced by the overexpression of *ScCaM*. *AtSTM* is a specific regulatory factor of the shoot apical meristem and plays an important role in shoot apical meristem formation during embryogenesis [31]. *AtSTM* has the function of formation and maintenance of stem cell population and floral and carpel formation [31–33]. It may be inferred that the overexpression of the allogenic *ScCaM* gene inhibits the expression of *AtSTM*, leading to the phenomenon of multiple-tillering in transgenic *A. thaliana*. Taken together, these results indicate that *ScCaM* acts as an age-dependent regulator. However, the mechanism of how *ScCaM* regulates the growth and development of plants still requires further study.

5. Conclusions

In the present study, one CaM gene was isolated from a *Saccharum* spp. hybrid (ROC22). The protein sequence of *ScCaM* was analyzed. The phylogenetic tree indicated that *ScCaM* has a closer relationship with CaMs from Poaceae. A qRT-PCR demonstrated the involvement of *ScCaM* in response to NaCl, CrCl\(_3\), SA, and MeJA stresses. Subcellular localization showed that *ScCaM* was located in the plasma membrane and nucleus. In addition, the overexpression of *ScCaM* was found to have an impact on the growth and development of *A. thaliana*. Taken together, although the mechanism of *ScCaM*-regulated growth and development still needs further research, the present study provides novel insights into the expression and function of *ScCaM* in sugarcane and offers useful clues for the further verification of its function in the future.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112153/s1: Table S1: Primers used in this study; Table S2: Accession numbers used in sequence and phylogenetic analysis; Table S3: The ANOVA analysis of *ScCaM* under various stresses; Table S4: The ANOVA analysis of *ScCaM* and *AtSTM*; Table S5: The ANOVA analysis of the expression of genes (*AtBRC1*, *AtCLV*, *AtWUS*, and *AtMAX*); Figure S1: The transcripts of *AtBRC*, *AtCLV*, *AtWUS*, and *AtMAX* in the *Arabidopsis thaliana* of transgenic line (T3-5) and the wild type (*Col-0*). *AtACT2* was used for normalization of the transcript levels.

Author Contributions: J.L. (Jinxian Liu), C.Z. and J.L. (Jun Luo) conceived, designed, and initiated the project. J.L. (Jinxian Liu), G.W. and X.F. prepared materials. J.L. (Jinxian Liu), C.Z., G.W. and X.F. performed experiments and contributed to data analysis and validation. J.L. (Jinxian Liu), J.L. (Jun Luo), W.S. and Y.Q. drafted the manuscript. J.L. (Jinxian Liu), J.L. (Jun Luo), W.S. and Y.Q. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key R&D Program of China (2019YFD1000500 and 2018YFD1000503), the National Natural Science Foundation of China (31871688, 31671752, 31101196 and 31340060), the Natural Science Foundation of Fujian Province, China (2018J01470 and 2015J06006), Scientific research projects of introducing talents in Wuyi University (YJ202109), and China Agriculture Research System of MOF and MARA (CARS-17). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data Availability Statement: The data supporting the conclusions of this article are within the paper.

Acknowledgments: We are grateful to the reviewers for their helpful comments on the original manuscript. We would like to thank the editors for their efficient work.

Conflicts of Interest: The authors declare no conflict of interest.
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