Genome-wide identification and expression analysis of calmodulin-binding transcription activator genes in banana under drought stress

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ABSTRACT

Banana (*Musa acuminata*) is an important economical food crop, cultivated worldwide depending on suitable climatic conditions. Its production is hindered because of several biotic and abiotic stresses. Drought stress is one of the main factors, which affect banana yield. To survive under water deficit conditions, plants respond by change in expression of many genes. Calmodulin-binding transcription activator (CAMTA) is a small transcription factor (TF) family with broad range of functions involved in different environmental stress responses including drought. Despite the importance of this family, no comprehensive data is available about this TF family in banana. In this study, five *Musa acuminata* CAMTA (MuCAMTA) TFs were identified in banana genome. One-month old banana plants cv. Basrai, were subjected to drought stress for twelve days by complete withholding of water. Plants kept for control were watered regularly. Photosynthetic pigments and total RNA was extracted from leaves of drought stressed and control plants. The expression pattern of *MuCAMTA* genes was identified by qRT-PCR. Drought resulted decrease in chlorophyll pigments. All *MuCAMTA* genes except *MuCAMTA2* showed upregulation under drought stress. *MuCAMTA1* showed highest expression (40 fold) under drought stress. *MuCAMTA1* could be an ideal candidate to enhance drought stress tolerance in banana plants.

1. Introduction

Plants are sessile organisms, so they are frequently exposed to wide range of biotic and abiotic stresses during their life cycle. These stresses can have severe impacts on plant physiology which affects plant growth and ultimately its yield (Danquah et al., 2014). Drought, salinity, low and high temperatures are the major abiotic stresses. To survive under these unfavorable conditions plants have developed different kinds of defense mechanisms to avoid or tolerate these extreme conditions (Bartels and Sunkar, 2005). Better understanding of these mechanisms can help in development of stress tolerant crops. Drought is one of the important factors which negatively affects crop yield globally (Singh and Laxmi, 2015). Because of climatic changes, plants could suffer from drought stress for long durations. To tackle present day and to get ready for future there is dire need to develop crops which can withstand long term water shortage.

Plants activate different kinds of signaling mechanisms in response to drought stress (Umezawa et al., 2006). These drought responsive regulatory networks have been explored in many plant species which helped in development of drought tolerant transgenic plants (Todaka et al., 2015). TFs are important components which regulate the expression of stress responsive genes under drought stress conditions

(Joshi et al., 2016; Nakashima et al., 2014). Calmodulin (CaM)-binding transcription activator (CAMTA), also referred as signal response (SR) proteins is an important TF family which belongs to Ca^{2+}/CaM-binding transcription factors (Bouché et al., 2002; Reddy et al., 2000). Calcium (Ca^{2+}) acts as secondary messenger in plants and shows different response to different environmental stresses (Reddy et al., 2011). Many Ca^{2+} binding proteins translate Ca^{2+} signatures into different biochemical and molecular pathways (Evans et al., 2001). CaM are the main sensor for change in Ca^{2+} concentration in plants. Mechanism of Calcium responses in nuclei have not been discovered yet but it showed that cytosolic Ca^{2+} and nucleic Ca^{2+} respond to Ca^{2+} changes independently (Pauly et al., 2000). CAMTAs are exclusively present in multicellular eukaryotes and are highly conserved (Bouché et al., 2002; Finkler et al., 2007). CAMTA TF contain CG-1 DNA binding domain, TIG domain, ankyrin (ANK) repeat domain and IQ motifs (CaM-binding domain) (Bouché et al., 2002).

CAMTA TFs regulate expression of many downstream genes involved in stress tolerance in plants (Janiak et al., 2016). Members of this gene family showed altered expression in plants in response to drought, extreme heat, cold, salinity, UV, wounding, ABA and salicylic acid (Pandey et al., 2013; Yang and Poovaiah, 2002). In *Arabidopsis thaliana camta* mutant plants showed enhanced drought susceptibility

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compare to wild type plants (Pandey et al., 2013). Role of CAMTA TF family has been explored in response to different biotic and abiotic stress conditions in other plants like potato (Zhao et al., 2013), tomato (Yang et al., 2012), medicago (Yang et al., 2015), maize (Yue et al., 2015), oilseed rape (Rahman et al., 2016a), *Populus trichocarpa* (Wei et al., 2017) and grapevine (Shangguan et al., 2014). In drought stress conditions, members of CAMTA family are involved in regulation of different drought responsive genes like ethylene-responsive element binding factor 13 (ERF13), C-repeat/DRE binding factor 2 (CBF2) and WRKY33 (Pandey et al., 2013). This information indicates the possible involvement of CAMTA TFs role in drought stress tolerance. Despite the importance of this TF family, it is still unexplored in many plant species including banana. Banana is the most consumed fruit worldwide and belongs to the family Musaceae (Langdon, 2008). Banana is grown in more than one hundred countries and is staple food in many parts of the world. However, its production is facing different biotic and abiotic stresses globally (Ploetz et al., 2015; Ravi et al., 2013). Scientists around the world have been mostly focusing on biotic constraints in banana but abiotic stresses like drought, salinity and heat did not gain much attention from research community. Among abiotic stresses, drought is one of the most conspicuous outcomes of global climatic change posing a serious threat to crop production and food security. Banana plants require regular water supply and are more prone to drought stress because of large leaf area used for transpiration and absence of true roots (Ravi et al., 2013). It is important to identify genes involved in drought stress tolerance in banana plants, which could be used to enhance drought stress tolerance in banana plants. Because of the involvement of CAMTA TFs in drought stress in model plants like Arabidopsis, it is interesting to explore their gene expression changes under drought stress conditions in banana plants to identify candidate genes which could be used in future to develop drought tolerant transgenic banana plants.

2. Material and methods

2.1. Identification of MuCAMTA TFs

Complete proteome of banana (*Musa acuminata* version 2) and *Arabidopsis thaliana* were downloaded from the National Center for Biotechnology Information (NCBI). BLAST software (Standalone version 2.4.0+) was also downloaded from NCBI. CAMTA protein sequences of *Arabidopsis thaliana* were downloaded from The Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/). To identify orthologs in banana a reciprocal BLASTP algorithm search was done between banana whole proteome sequences and *Arabidopsis thaliana* proteome sequences. Orthologs were detected based on reciprocal BLAST hits defined as having > = 80% query coverage, E-value < 10^{-6}, and > = 50% identity. From the set of identified orthologs, a further BLAST search was performed against the arabidopsis CAMTA TF family using already mentioned BLAST parameters. An in-house Python script was used to eliminate redundant protein data. Non-redundant sequences with ≥ 80% query coverage were selected as candidate genes for MuCAMTA TFs. After identification of MuCAMTA protein sequences, the putative MuCAMTA TFs were further confirmed for the presence of conserved domains with the help of NCBI conserved domains database (CDD) (Marchler-Bauer et al., 2017) and SMART tool (Letunic and Bork, 2018). The protein sequences lacking CAMTA specific domains were discarded. The predicted molecular mass and isoelectric points (pI) of identified MuCAMTA proteins were determined by ExPASy tool (Bjellqvist et al., 1993).

2.2. Gene structure

The genomic architecture of identified *MuCAMTAs* representing exons/introns was identified with the help of gene structure display server (GSDS) version 2.0 (Hu et al., 2015). Genomic and CDS

sequences of *MuCAMTA* genes were downloaded from NCBI.

2.3. Plant material

Tissue cultured banana plants cv. Basrai were collected from National Agriculture Research Center (NARC) Islamabad, Pakistan and were grown in pots under controlled growth conditions of 16:8-hour day/night light cycle at 30 °C in plant growth room. After one month, banana plants with fully expanded leaves were subjected to drought stress by complete withholding water for twelve days. Plants kept for control sample were watered regularly. At the end of treatment, leaf samples were collected from both drought stressed and control plants for total RNA extraction and subsequent analysis. Three independent biological replicates were taken for this study.

2.4. Photosynthetic pigments

The chlorophyll and carotenoid pigments were extracted and measured as described by Wellburn (1994). Fresh leaf samples (about 0.3 g) were collected from control and drought stressed plants at end of experiment and were dipped in 10 mL of 80% acetone and were kept at 4 °C in dark for 5 days. After this the absorbance of the extracted material were taken at 470, 649 and 663 wavelengths.

2.5. RNA isolation and gene expression analysis

Total RNA was isolated from leaf samples of control and drought stressed plants by using Trizol reagent (Thermo Fisher Scientific, USA) following the manufacturer's instructions. The integrity of the extracted RNA was analyzed by 1% denaturing agarose gel. Good quality RNA samples were quantified by using nanodrop spectrophotometer. After RNA quantification, each sample was further processed with DNase 1 (Thermo Fisher Scientific, USA) to remove DNA contamination. The DNA free RNA sample was converted into cDNA using cDNA synthesis kit (Thermo Fisher Scientific, USA). Gene specific primers for qPCR were designed for the identified *MuCAMTA* genes by using NCBI primer designing tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and are listed in Table S1. qRT-PCR was carried out using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, USA). Actin was used as reference gene. Three independent biological replicates for both control and drought stressed plants were used to identify expression patterns of *MuCAMTA* genes by qRT-PCR. Relative fold change of target genes was determined by 2-ΔΔCt method as described by Livak and Schmittgen (Livak and Schmittgen, 2001).

2.6. Statistical analysis

Photosynthetic pigments and rqRT-PCR data was analyzed in excel spreadsheet by using *t*-test with a significance level set at P < 0.05. The data shown are the mean values ± SD of three replicates.

3. Results and discussion

3.1. Identification of CAMTA genes in Musa acuminata

Local reciprocal blast between *Musa acuminata* proteome and *Arabidopsis thaliana* proteome resulted in identification of 16,588 unique ortholog protein sequences with 80% query coverage at E-value of 10^{-6}. The protein sequences of six *Arabidopsis thaliana* CAMTA TFs downloaded from TAIR database (https://www.arabidopsis.org/) were used as query to find *Musa acuminata* CAMTA (MuCAMTA) TFs among retrieved 16,588 orthologs protein sequences. Local reciprocal blast against 16,588 unique protein sequences with 80% query coverage, 60% identity and 10^{-6} E-value resulted in identification of eighteen unique sequences. These sequences were confirmed for the presence of CAMTA TF specific domains with the help of NCBI conserved domains