Cloning and Expression Analysis of the Cassava MeHSF10 Gene Postprint

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Abstract

Heat shock transcription factor (HSF) is an important stress regulatory factor in plants. Numerous studies have demonstrated that HSF enhances plant adaptability to adverse conditions by regulating stress-related genes downstream of signaling pathways, such as improving tolerance to stresses including drought and oxidative damage. To investigate the function of HSF in cassava stress resistance and postharvest storage, this study employed cassava variety SC124 as material and cloned a cassava HSF family gene from cassava leaves via RT-PCR technology, designating it as MeHSF10. The results revealed: (1) The gene has a full length of 1,098 bp, encoding 365 amino acid residues, with a predicted protein relative molecular weight of 40.7 kD and a theoretical isoelectric point of 8.15; the subcellular localization of the protein is predicted to be nuclear. Protein sequence analysis indicated that MeHSF10 exhibits the highest similarity with JcHSF from Jatropha curcas and HbHSF from Hevea brasiliensis, at 80.31% and 90.54%, respectively. The protein sequence of MeHSF10 contains conserved domains characteristic of the HSF protein family, including DBD, HR-A Core, HR-B Core, insert sequences, and nuclear localization signal (NLS), suggesting that the protein encoded by MeHSF10 belongs to the HSFC family. (2) To analyze the expression pattern of MeHSF10 in different cassava tissues, its expression across 11 cassava tissues was examined; the results demonstrated that MeHSF10 is expressed in all cassava tissues, with the highest expression level in leaves. (3) Cis-element analysis of the MeHSF10 promoter sequence revealed the presence of elements such as ABA responsive motif, drought-induced motif, and light-responsive motifs. (4) Expression analysis further demonstrated that MeHSF10 can be significantly induced by drought and ABA treatment, and is also significantly upregulated during the postharvest physiological deterioration of cassava tubers. Collectively, these results suggest that MeHSF10 may participate in ABA-mediated cassava drought stress response and postharvest physiological deterioration of cassava tubers at the transcriptional level, thereby establishing a foundation for further functional investigation of its role

in cassava stress resistance and postharvest storage.

Full Text

Clone and Expression Analysis of MeHSF10 in Manihot esculenta

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Abstract

Heat shock transcription factors (HSFs) are crucial regulatory elements in plant stress responses. Numerous studies have demonstrated that HSFs enhance plant adaptability to adverse conditions by regulating downstream stress-responsive genes, thereby improving tolerance to drought, oxidative damage, and other environmental stresses. To investigate the function of HSF genes in cassava stress resistance and postharvest storage, we cloned a novel HSF family gene from cassava (Manihot esculenta Crantz) leaves using RT-PCR and designated it as *MeHSF10*. Our results revealed: (1) The full-length gene spans 1,098 bp, encoding 365 amino acid residues with a predicted molecular weight of 40.7 kDa and theoretical isoelectric point of 8.15. Subcellular localization prediction indicated nuclear targeting. Protein sequence analysis showed highest similarity with Jatropha curcas JcHSF (80.31%) and Hevea brasiliensis HbHSF (90.54%). The MeHSF10 protein contains conserved HSF domains including DNA-binding domain (DBD), HR-A Core, HR-B Core, insert sequences, and nuclear localization signal (NLS), confirming its classification as an HSFC family member. (2) Expression profiling across 11 cassava tissues demonstrated ubiquitous expression with highest levels in leaves. (3) Promoter element analysis identified ABA-responsive motifs, drought-induced elements, and light-responsive motifs. (4) Expression analysis confirmed significant induction of *MeHSF10* by drought and ABA treatment, as well as during postharvest physiological deterioration (PPD) of cassava tubers. These findings suggest that *MeHSF10* may participate in ABA-mediated drought stress responses and PPD at the transcriptional level, providing a foundation for further functional studies in cassava stress resistance and postharvest storage.

Keywords: heat shock transcription factor, MeHSF10, abiotic stress, postharvest physiological deterioration, expression analysis

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Introduction

Plants have evolved sophisticated defense mechanisms to cope with various environmental stresses during their long evolutionary history (Ohama et al., 2017). The heat shock response plays a critical role in plant responses to abiotic stress, with heat shock transcription factors (HSFs) serving as key regulatory elements. HSFs enhance plant adaptability to adverse conditions by modulating downstream stress-responsive genes, thereby improving tolerance to heat, drought, and oxidative damage (Guo et al., 2016; Zhou et al., 2018; Jiang et al., 2018). Different HSF genes confer stress tolerance across diverse plant species, such as *TaHSFA4a* enhancing heavy metal tolerance in wheat (Shim et al., 2009), *SlHsfA1* improving high-temperature survival in tomato (Scharf et al., 2012), *CarHsfB2* increasing drought resistance in Arabidopsis (Hao et al., 2016), and *AtHSFA6a*, *AtHSFA6b*, and *AtHSFA2* conferring tolerance to salt, low temperature, and osmotic stress (Miller & Mittler, 2006; Banti et al., 2010). Thus, HSF genes from various species consistently enhance plant resilience to multiple environmental challenges.

Cassava (*Manihot esculenta* Crantz) is a vital food crop widely cultivated in tropical and subtropical regions. In China, cassava cultivation covers 5.0×10 hectares with annual production of approximately 1.0×10^{1} kg and an output value exceeding 14 billion yuan, representing an important economic crop in southern China (Zhang et al., 2014). As a promising energy plant with significant potential for biofuel development, cassava is utilized for industrial starch production, fuel ethanol, and bio-based materials (Zhang et al., 2014; Hu et al., 2016; Yan et al., 2018). While cassava exhibits natural drought tolerance and adaptability to poor soils, its commercial potential is severely limited by postharvest physiological deterioration (PPD), which shortens storage life and hinders industrial processing (Zidenga et al., 2012; Zhang & Li, 2012; Xu et al., 2013). Currently, functional studies of HSF genes in cassava remain scarce (Wei et al., 2018; Yu et al., 2019). Therefore, cloning and expression analysis of cassava HSF genes is essential for elucidating their regulatory roles in stress responses

and PPD processes. In this study, we cloned a heat shock transcription factor gene *MeHSF10* from cassava transcriptome data, performed preliminary analysis of its protein sequence, conserved domains, and phylogenetic relationships, and examined its expression patterns under drought stress, ABA treatment, and PPD conditions to provide insights into its functional mechanisms.

1.1 Plant Materials

The cassava cultivar SC124 (*Manihot esculenta* cv. SC124) was obtained from the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences. The Plant RNA Extraction Kit (Cat. No. DP437) was purchased from Tiangen Biotech, and the cDNA Reverse Transcription Kit (Cat. No. K1622) was obtained from Fermentas. PCR primers were synthesized by Shanghai Sangon Biotech.

1.2 Stress Treatments

Cassava stems were cut into sections containing 3-4 buds and planted in a substrate mixture of vermiculite and nutrient soil (1:1, V/V). After approximately 60 days of growth, uniform seedlings were selected for experiments. For drought simulation, plants were treated with 20% (W/V) PEG-6000, while control plants received water. Leaf samples were collected at 0, 3, 5, and 7 days post-treatment, immediately frozen in liquid nitrogen, and stored at ultra-low temperature. For ABA treatment, plants were irrigated with 100 M ABA solution, with leaf samples collected at the same time points and processed identically. For PPD analysis, 10-month-old cassava storage roots were incubated at 25°C with 70% relative humidity in darkness, and samples were collected at 0, 6, 12, and 48 hours, frozen in liquid nitrogen, and stored. All treatments included three biological replicates.

1.3 Gene Cloning

Total RNA was extracted using the RNA extraction kit and reverse-transcribed using the cDNA synthesis kit. Based on the cassava homologous sequence (Manes.02G087400.1), primers were designed (5' -CTAAAAGCCACCACCTAAAAGCG-3'and 5'-ATGAGCAAAAAAGAAAAAAAAG-3') to amplify *MeHSF10* from leaf cDNA. The PCR product was ligated into the pMD-18T vector, transformed into *E. coli*, and positive clones were sequenced.

1.4 Bioinformatics Analysis

Homologous protein sequences were identified using BLASTp in NCBI. Subcellular localization was predicted with Plant-mPLoc software. Conserved domains were analyzed using the NCBI-CDD database. Theoretical isoelectric point and

molecular weight were calculated via ExPASy ProtParam. Sequence alignment was performed using DNAMAN6 software. Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method in MEGAX. Primers were designed with Primer 5.0 software. Promoter elements were analyzed using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

1.5 Expression Analysis

RNA extraction, library construction, and sequencing were performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. using the Illumina GAII platform (Illumina, San Diego, CA, USA). Adapter sequences and low-quality reads were removed using FASTX-toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Clean reads were aligned to the cassava reference genome (version 4.1) using TopHat 2.0 (Trapnell and Pachter, 2009), and transcriptome assembly was performed with Cufflinks (Trapnell et al., 2012), retaining transcripts present in at least two samples. Gene expression levels under ABA treatment, PEG treatment, and PPD were quantified as FPKM (Fragments Per Kilobase of transcript per Million mapped reads).

2.1 Cloning of MeHSF10 Gene

Using *Arabidopsis thaliana* AtHSF8 protein sequence (Accession: AT3G24520) as a query, BLASTp search in the Phytozome database identified a highly similar cassava sequence (Manes.16G116200.1). Primers designed based on this sequence amplified a 1,098 bp fragment [Figure 1: see original paper], encoding 365 amino acids, designated as *MeHSF10*. Sequence comparison revealed a single synonymous SNP between the cloned gene and the reference sequence [Figure 2: see original paper]. The *MeHSF10* gene contains one intron and two exons. The predicted protein formula is C H N O S , with a molecular weight of 40.7 kDa, theoretical pI of 8.15, and an instability index of 60.57, classifying it as an unstable protein. Subcellular localization prediction indicated nuclear targeting. Conserved domain analysis confirmed the presence of HSF family domains [Figure 3: see original paper], verifying that the cloned gene encodes a cassava HSF protein.

2.2 Sequence Alignment and Phylogenetic Analysis

BLASTp analysis using the MeHSF10 protein sequence identified highly homologous sequences, with highest similarity to *Hevea brasiliensis* HbHSF (XP_021643078.1, 90.54%) and *Jatropha curcas* JcHSF (XP_020535025.1, 80.31%). Multiple sequence alignment revealed conserved HSF domains [Figure 4: see original paper], including a highly conserved DNA-binding domain (DBD) at amino acid residues 6-100, HR-A Core, HR-B Core and insert sequences at residues 170-210, and a nuclear localization signal (NLS) at residues 227-242, confirming MeHSF10 as an HSFC family member. Phylogenetic analysis

showed MeHSF10 clustering most closely with rubber tree HbHSF [Figure 5: see original paper].

2.3 Tissue-Specific Expression Analysis

Expression data from 11 untreated cassava tissues were obtained from the Cassava Atlas database (shiny.danforthcenter.org/cassava_atlas/) to investigate *MeHSF10* expression patterns across different organs: leaf, midvein, petiole, stem, lateral bud, shoot apical meristem (SAM), storage root (SR), fibrous root (FR), root apical meristem (RAM), organized embryogenic structure (OES), and friable embryogenic calli (FEC). Results showed differential expression across tissues, with highest levels in leaves and lower expression in less differentiated tissues including FEC, RAM, and SAM [Figure 6: see original paper].

2.4 Expression Analysis Under Various Stress Conditions

Promoter analysis of the 1,500 bp sequence upstream of the *MeHSF10* start codon identified one drought-induced MBS element and three ABA-responsive ABRE elements . Expression analysis under ABA treatment and simulated drought stress revealed significant upregulation of *MeHSF10*, with maximum induction of 2.3-fold and 2.4-fold, respectively [Figure 7: see original paper].

To investigate the relationship between *MeHSF10* and PPD, expression was analyzed during postharvest physiological deterioration. *MeHSF10* expression was strongly induced during PPD, showing no significant change at 6 h, peaking at 12 h with a 4.3-fold increase, and remaining elevated at 48 h compared to 0 h [Figure 8: see original paper].

Discussion

Heat shock transcription factors comprise multiple gene family members in nature, containing distinct conserved domains including DNA-binding domain (DBD), oligomerization domain (OD), and nuclear localization signal (NLS). Based on OD characteristics, HSFs are classified into HSFA, HSFB, and HSFC families (Guo et al., 2016). HSF family size varies among plants, with 21 members in Arabidopsis, 25 in rice, 25 in maize, and over 56 in wheat, all containing conserved HSF domains that have been functionally characterized (Guo et al., 2008; Mittal et al., 2009; Lin et al., 2011; Scharf et al., 2012; Xue et al., 2014). However, HSF research in cassava remains limited. The *MeHSF10* gene isolated in this study spans 1,098 bp and encodes 365 amino acids. Sequence analysis confirmed the presence of conserved HSFC domains (Li et al., 2017), while phylogenetic analysis revealed close relationships with rubber tree HbHSF and Jatropha JcHSF.

HSF genes exhibit diverse expression patterns across plant species and tissues. Some HSFs are expressed in all rice tissues, while others like HSF9 are seed-

specific in sunflower and Arabidopsis (Almoguera et al., 2002; Scharf et al., 2012). Tomato HSFA2 shows elevated expression in pollen compared to other floral tissues (Guo et al., 2016). These tissue-specific expression patterns reflect functional specialization. The *MeHSF10* promoter contains multiple light-responsive elements , and its highest expression in leaves suggests potential involvement in photosynthesis-related processes.

Promoter analysis revealed drought-induced MBS elements and ABRE motifs in the *MeHSF10* promoter. Consistently, *MeHSF10* expression was significantly induced by both drought stress and ABA treatment. In Arabidopsis, AtHsfA9 enhances drought tolerance through ABA signaling pathways (Guo et al., 2008), while overexpression of *AtHSFA2* and *AtHSFA8* improves salt and osmotic stress tolerance. Similarly, overexpression of *OsHSF29* and *OsHSF17* enhances stress adaptation in rice (Jin et al., 2013). These findings suggest that *MeHSF10* may respond to drought stress through ABA-dependent pathways, though the specific downstream target genes remain to be identified. The strong induction of *MeHSF10* during PPD implicates its involvement in cassava tuber deterioration. Previous studies have linked HSFs to oxidative stress responses; for example, *OsHsfC2a* and *OsHsfA5* are crucial for ROS sensing and accumulation in rice (Miller et al., 2008; Mittal et al., 2009), while overexpression of *PeHSF* in tobacco enhances abiotic stress tolerance by modulating leaf ROS homeostasis (Shen et al., 2013). Therefore, we hypothesize that *MeHSF10* may influence PPD through regulation of oxidative stress. However, the precise molecular mechanisms of *MeHSF10* involvement in cassava tuber deterioration require further investigation. These findings provide a theoretical foundation for improving cassava stress resistance and delaying postharvest deterioration. Future studies will focus on functional characterization to validate the role of *MeHSF10* in drought tolerance and PPD mitigation.

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