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Comparative Analysis of C-repeat Binding Factors (CBFs) in Tomato and Arabidopsis

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HIGHLIGHTS

- This study focused on the investigation CBF genes in Arabidopsis and tomato.

- The different cis-regulatory elements were observed in promoter sites.

- SlCBF1 and AtCBF1 genes showed differential expression under cold stress.

- AtCBF1 is more induced than SlCBF1 under cold stress.

Abstract: Cold stress is one of the limiting factors of plant production that plants use different mechanisms for cold tolerance. CBF genes play critical role to regulate the cold responsive genes. To better understand of CBF gene functions, the tomato-CBFs and Arabidopsis-CBFs were evaluated using bioinformatics tools, and finally the expression patterns of SlCBF1 gene were analyzed under 10 and 4°C in two contrasting tomato species (*Solanum lycopersicum* and *S. habrochaites*). The different cis regulatory elements were observed in promoter region of SlCBF1 and AtCBF1 genes, and ICE1, COR and HOS1 proteins exhibited high interaction with CBFs. The results of Real time PCR of SlCBF1 exhibited that under 10 and 4 °C, SlCBF1 was down regulated in cold sensitive tomato genotype while it was slightly up-regulated in cold tolerant genotype at 4 °C. The results showed that the SlCBF1 and AtCBF1 genes have differential expression in cold stress.

Keywords: Cold stress; CBF genes; Real time PCR; Tomato; Bioinformatics analysis.

INTRODUCTION

Environmental stresses such as low temperature (<18°C), high temperature, drought and high salinity have negative effects on plant growth, development and performance [1, 2]. To adapt to unfavorable conditions such as cold stress, plants use the different mechanisms including molecular and physiological responses [3]. Transcription factors (TFs) as a molecular response have key role to regulate the expression patterns of target genes under

biotic and abiotic stresses [4]. The AP2/ERF superfamily is a large TF family that is involved in the response to environmental stresses [5]. C-repeat binding factors (CBFs), also known as DREB, are members of AP2/ERF superfamily that play a fundamental role in regulation of cold-responsive genes and cold acclimation [6, 7]. During cold stress in Arabidopsis, CBF1/DREB1B, CBF3/DREB1A, and CBF2/DREB1C are induced, and they bind the regulatory CRT/DRE element with conserved core sequence (5'-CCGAC-3') of promoter region in cold-regulated genes [7, 8]. Previous studies illustrated that CBFs are induced under low temperature in barley [9], rice [10], Arabidopsis [11, 12] and wheat [13].

Tomato (*Solanum lycopersicum*) is one of the largest angiosperm genera and includes annual and perennial plants that most genotypes of tomato are sensitive to low temperature [14]. Three CBFs (*SlCBF*1, 2 and 3) were identified in tomato that *SlCBF1* is induced in low temperature [15]. Recently, Li et al. generated slcbf1 mutant lines in tomato by the CRISPR/Cas9 system that the abscisic acid, methyl jasmonate, zeatin riboside and protein contents were decreased in slcbf1 mutants and wild type showed more cold resistance than mutant lines [16]. However, the overexpression of *SlCBF1*in tomato could not increase the cold tolerance, but *SlCBF1* increased the cold stress tolerance in Arabidopsis transgenic plants [15]. It seems that the function of *SlCBF1* in tomato is different from other plant species such as Arabidopsis, and its role is still unknown under cold stress. To understand the role of *SlCBF1* in tomato during low temperature stresses, the expression patterns of *SlCBF1* in tomato genotypes (cold sensitive and cold tolerant genotypes) were analyzed at 10˚C and 4 ˚C and also the Arabidopsis-CBFs and Tomato-CBFs were compared using bioinformatics tools. The results of this research provide the new information of SlCBF1-regulatory mechanisms during the cold stresses.

MATERIAL AND METHODS

Sequence analysis

The protein sequences of CBF in tomato, Arabidopsis, wheat, barley, rice and potato were retrieved using NCBI database [17]. The some physicochemical characteristics of studied proteins such as molecular weight (MW) and isoelectric point (pI) were predicted using ProtParam tool [18], and the subcellular location of CBF proteins was predicted by Plant-mPLoc tool [19]. The STRING database [20] was used to predict the protein-protein interaction of tomato-CBFs and Arabidopsis-CBFs.

Phylogenetic analysis

The amino acid sequence of CBF proteins in tomato, Arabidopsis, potato and some important monocots such as wheat, barley and rice was used to construct the phylogenetic analysis using neighbor-joining method of MEGA7 software [21].

Cis-acting regulatory elements analysis

To identify the cis-regulatory elements in promoter regions, the 1000bp of upstream the start codon of *SlCBF1* and *AtCBF1* were analyzed using Plant CARE [22].

Microarray analysis

The published data of genes expression profile of Arabidopsis were used to consider the expression patterns of AtCBF1-3 genes under abiotic stresses. The microarray data of AtCBF1-3 genes under cold, drought and salt stresses were obtained using the affymetrix Arabidopsis ATH1 genome array (10615 samples) from Genevestigator database [23].

Plant materials and growth conditions

Seeds of *Solanum lycopersicum* cv. Moneymaker (as cold sensitive) and *S. habrochaites*, LA1777 (as cold tolerant) were sown in 18 soil plates at 23±1ºC under 14h photoperiod duration. After six weeks, the 12 plates of tomato seedlings were transferred to two different growth chambers at set-point temperatures of 10±1°C and 4±1°C and 6 plates remained at 23±1ºC. After three days, the whole shoots of each plate were collected and stored in liquid nitrogen and transferred to -80ºC.

RNA extraction and cDNA preparation

The leaves from three individual tomato seedlings were powdered in liquid nitrogen and then the total RNA was extracted by RNX TM -Plus (Sinaclon). The quantity and quality of extracted RNA were determined using a Nano Photometer (Implen N50). Reverse transcription was carried out using 1 μg total RNA treated with RNase-free DNase I (Thermo Scientific) and reverse transcriptase (Roche, Germany) according to instructions of manufacture. The primers for *SlCBF1* (F: 5´- CCTGCTTCCTCCAACTCTAAA -3´ and R: 5´-CTCATCCACGAAGTCACTACTC   -3´)   and   *EF-1-α*   (F: 5´-GGAACTTGAGAAGGAGCCTAAG -3´ and R: 5´- CAACACCAACAGCAACAGTCT -3´) as reference gene were designed and evaluated using primer3 plus [24].

Real time PCR

The real time PCR was run with Applied Biosystems StepOne TM using RealQ Plus 2x Master Mix Green high ROX TM (Ampliqon) according to instructions of manufacture. The conditions of real time PCR were combined: 95ºC for 10 min, followed by 35 cycles at 95ºC for 15s and 61ºC for 20s. The melting curve for each sample was carried out after 35 cycles. The relative expression patterns of *SlCBF1* were evaluated using the $2^{-\Delta\Delta Ct}$ method [25].

RESULTS and DISCUSSION

Physicochemical properties of CBF proteins

The molecular weight (MW) and isoelectric point (pI) of CBF proteins in tomato, Arabidopsis, wheat, barley, rice and potato were predicted as shown in table 1. The results revealed that the length of studied CBFs ranged from 205 (tomato-CBF3) to 257aa (potato-CBF2). The pI value of CBFs in dicots plants (tomato, Arabidopsis and potato) was less than monocots plants (wheat, barley and rice) as the rice-CBF showed the highest value (10.18) and potato-CBF2 had the lowest value (4.54). The prediction of CBFs-subcellular location exhibited that the cellular location of Arabidopsis-CBF1-3 was in nucleus while the subcellular location of tomato-CBF1 was predicted in cytoplasm and nucleus.
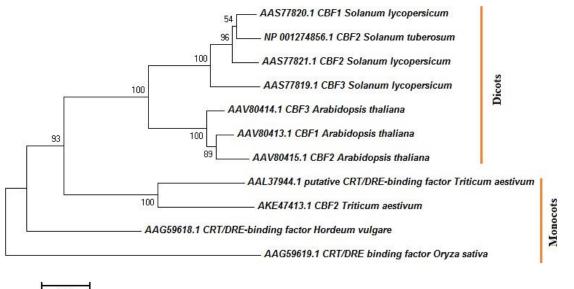
Phylogenetic analysis of CBFs

To understand the phylogenetic relationship between CBFs in monocots and dicots plants, the phylogenetic tree was constructed based on multiple alignments of amino acid sequences of CBFs in tomato, Arabidopsis, wheat, barley, rice and potato (Fig. 1). According to the evolution analysis, the CBFs of dicots plants were visibility separated from monocots. The CBF proteins from tomato showed closer relationships to CBF3 protein from potato. Also, CBF1 and CBF2 in tomato exhibited close similarity as well as CBF1 and CBF2 proteins from Arabidopsis.

Table 1- Properties of CBF proteins in Arabidopsis, tomato, potato, wheat, barley and rice

Gene accession number	Gene name	Organism	Length (aa)	MW (KDa)	pI	Predicted location(s)
AAS77820	CBF1	*Solanum lycopersicum*	210	23.40	5.23	Cyto*. Nucleus.
AAS77821	CBF2	*Solanum lycopersicum*	220	24.60	5.33	Nucleus.
AAS77819	CBF3	*Solanum lycopersicum*	205	23.06	5.48	Cyto. Nucleus.
AAV80413	CBF1	*Arabidopsis thaliana*	213	23.82	5.08	Nucleus
AAV80415	CBF2	*Arabidopsis thaliana*	216	24.27	5.00	Nucleus
AAV80414	CBF3	*Arabidopsis thaliana*	216	24.25	5.08	Nucleus
NP_001274856	CBF2	*Solanum tuberosum*	257	28.45	4.54	Cyto. Nucleus
AAL37944	CBF	*Triticum aestivum*	212	23.34	7.78	Cyto. Nucleus
AKE47413	CBF2	*Triticum aestivum*	225	25.14	5.11	Cyto. Nucleus
AAG59618	CBF	*Hordeum vulgare*	249	26.33	5.33	Cyto. Nucleus
AAG59619	CBF	*Oryza sativa*	253	27.67	10.18	Cyto.

Cyto: cytoplasm

Figure 1- Phylogenetic analysis of CBF proteins using neighbor-joining method

Protein-protein interactions in CBFs

The interaction network of CBF genes in tomato and Arabidopsis revealed that Solyc03g082820 (uncharacterized protein) just had an interaction with CBF1-3 from tomato while Arabidopsis-CBF1-3 exhibited a high interaction with each other (Fig. 2). Arabidopsis is model plant that its molecular aspects have been extensively investigated more than tomato. CBF1 from tomato had an interaction with THM18 (also known as SlMYB14) which is a member of R2R3MYB gene family that involves in plant response to environmental stresses. The some key cold-response proteins such as ICE1, COR15A and HOS1 exhibited high interaction with Arabidopsis-CBF1-3. ICE1 is an inducer of CBF genes in Arabidopsis that it binds to MYB elements of CBFs promoter under cold stress [26]. ICE1 is upstream of cold responsive pathway and regulates the key cold regulated genes such COR15A and CBF genes [26, 27]. HOS1 was observed as a member of CBFs interaction

network that interacts with ICE1. HOS1 is a negative regulator of cold responses and it involve in ubiquitination and degradation of ICE1 as an E3 ligase [28].
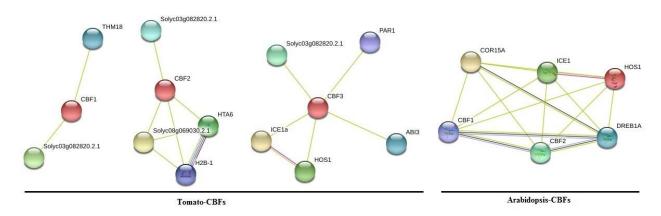

Figure 2- Proteins interaction network of CBF proteins in tomato and Arabidopsis

Promoter analysis

Cis-regulatory elements, such as promoters and enhancers, control physiology and development by regulating gene expression [2, 29]. The different cis-regulatory elements were found in upstream sequences (1000bp) of *SlCBF1*and *AtCBF1* that they involve in biotic and abiotic stresses (Fig. 3). The GARE motif (gibberellin-responsive element), CGTCA-motif (cis-acting regulatory element involved in the MeJA-responsiveness) and TC-rich repeats (cis-acting element involved in defense and stress responsiveness) were distributed within the regulatory region of *SlCBF1* and *AtCBF1* genes. Some key regulatory elements such as HSE (cis-acting element involved in heat stress responsiveness) and MYS (MYB binding site involved in drought-inducibility) were observed in upstream of *SlCBF1*. The ABRE and AuxRR which involve in abscisic acid (ABA) and auxin hormones signaling respectively were found in promoter region of *AtCBF1*. ABRE is an ABA response element that plays critical role in abiotic stresses [30]. However *CBF1* is expressed by ABA independent pathway [26]. The identification of cis-regulatory DNA elements responsive to stress is important to determine gene regulatory mechanisms under various stresses [2, 31]. The present result revealed that *SlCBF1* and *AtCBF1* have different regulatory elements in their promoter region which caused different-induction patterns.
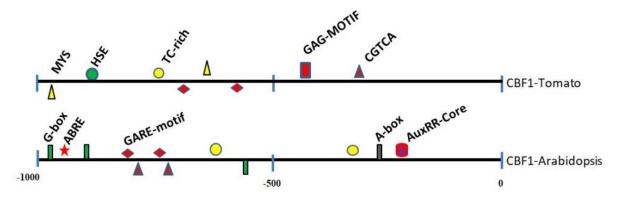

Figure 3- Distribution of cis-acting regulatory elements in the 5′ regulatory sequences of CBF1in tomato and Arabidopsis

Expression patterns of *SlCBF1* gene in tomato genotypes

To more understand the role of *SlCBF1* under cold stress, the expression patterns of *SlCBF1* gene were evaluated at 10 and 4˚C in sensitive and tolerant tomato genotypes (Fig. 4). The *SlCBF1* gene exhibited the different expression patterns in tomato genotypes whereas *SlCBF1* was down regulated in Moneymaker (as cold sensitive) at 10 and 4˚C after 3 days but it was up-regulated at 4˚C in LA1777 (as cold tolerant genotypes). CBF1 is key transcription factor that involve in plant response to low temperature, and drought stress [3]. Zhang et al. and Liu et al. also reported that *SlCBF1* was induced under cold stress (4˚C) [15, 32]. It is not surprising to see that *SlCBF1* gene was not induced at 10 ˚C in cold tolerant genotype that probably *SlCBF1* is not induced in low temperature when it decreased to 4 ˚C, *SlCBF1* was stimulated. To compare the *SlCBF1* with Arabidopsis-CBF1-3, the expression profiles of *AtCBF1*, *AtCBF2* and *AtCBF3* were evaluated in cold, drought and salt stresses using Arabidopsis microarray data. *AtCBF1*, *AtCBF2* and *AtCBF3* were highly expressed under cold stress (Fig. 5). *AtCBF1* gene was found to be more expressed in cold stress than *AtCBF2* and *AtCBF3* while it was less induced in drought and salt stresses than others. In Arabidopsis, CBF1 is an early response and induces the CBF2 and CBF3 that involve stimulating the cold responsive genes [33]. It seems that *AtCBF1* is main transcriptional activator during cold temperature and overexpressed *AtCBF1* could increase the cold tolerance [34]. According to results of gene expression patterns, *AtCBF1* is more involved to induce the key cold-regulated genes than *SlCBF1.* It seems that the regulatory mechanisms of tomato are probably different from Arabidopsis
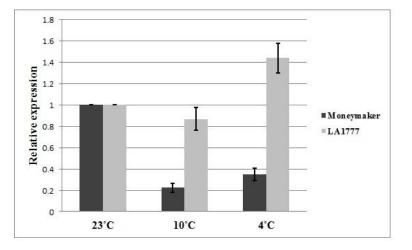

Figure 4– The *SlCBF1* expression patterns under cold stresses (10 and 4˚C) after 3 days in Moneymaker (as cold sensitive genotype) and LA1777 (as cold tolerant genotype).
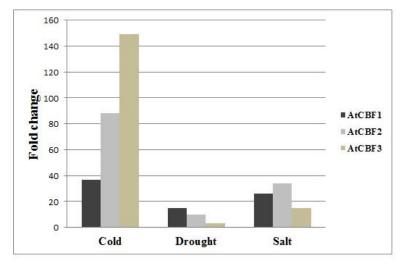
Figure 5– The expression profile of Arabidopsis-CBF1-3 under cold, drought and salt stresses based on microarray data using Genevestigator database

CONCLUSION

In this study, CBF1-3 from Arabidopsis and tomato were compared based on properties of protein sequence, phylogenetic analysis, interaction network and types of cis regulatory elements in promoter region. The results showed that tomato-CBFs are similar to Arabidopsis-CBFs based on amino acid sequences but they have different cis regulatory elements in promoter sites which could effect on their regulatory mechanisms. To better understand the function of CBF genes, the expression patterns of *SlCBF1* were considered at10 and 4˚C in two contrasting tomato genotypes using qPCR that *SlCBF1* exhibited differential expression in tomato genotypes. Present study revealed that CBF genes from Arabidopsis are more involved in cold stress than tomato-CBFs. The different factors such as promoter sequences and protein-protein interactions could affect on CBF functions.

Conflicts of Interest: The author declares that i have no conflict of interest.

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