FEBS Letters 583 (2009) 3329–3334

FEBS Letters

journal homepage: www.FEBSLetters.org

Ethylene and cold participate in the regulation of *LeCBF1* gene expression in postharvest tomato fruits

Danying Zhao [a,b,1], Lin Shen [a,c,1], Bei Fan [d], Mengmeng Yu [a], Yang Zheng [a], Shengnan Lv [a], Jiping Sheng [a,*]

[a] College of Food Science and Nutritional Engineering, China Agriculture University, Beijing 100083, China
[b] Beijing Xicheng District Disease Control and Prevention Center, Beijing 100011, China
[c] Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China
[d] Institute of Agro-Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, China

ARTICLE INFO

Article history:
Received 6 July 2009
Revised 3 September 2009
Accepted 3 September 2009
Available online 18 September 2009

Edited by Ulf-Ingo Flügge

Keywords:
Tomato
Postharvest fruit
Cold tolerance
Cold storage
Lycopersicon esculentum putative transcriptional activator *CBF1*

ABSTRACT

C-Repeat/dehydration-responsive element binding factor (*CBF*) is a transcription factor regulating cold response in plants, of which little is known in fruits. We showed a double-peak expression pattern of *Lycopersicon esculentum* putative transcriptional activator *CBF1* (*LeCBF1*) in mature green fruit. The peaks appeared at 2 and 16 h after subjection to cold storage (2 °C). The second peak was coincident with, and thus caused by a peak in endogenous ethylene production. We showed that *LeCBF1* expression was regulated by exogenous ethylene and 1-methylcyclopropene, and was not expressed without cold induction. *LeCBF1* expression was different in the five maturation stages of fruits, but expression peaked at 2 h at all stages.

1. Introduction

Plants have evolved various protective mechanisms to cope with cold stress. However, some species from tropical regions, such as tomato, are unable to tolerate freezing and suffer chilling injury when exposed to a cold (0–12 °C) environment. Transcription factors play an important role in the cold resistant mechanisms, regulating downstream cold-responsive genes to trigger resistant processes to cold [1,2]. *Arabidopsis CBF* (C-repeat/dehydration-responsive element binding factor, *DREB1*) transcription factors recognize the C-repeat/DRE DNA regulatory elements of *COR* (cold-regulated) genes and bind to them, thus play a prominent role in regulating the cold acclimation response [3,4]. Many studies have shown that *CBF* genes widely exist in cold tolerant- and sensitive-plants, with conserved nucleic acid sequences and functions [5,7,8]. Two aspects of this research have provided convincing evidence for the crucial function of *CBF*. Firstly, employment of array-based transcript profiling technology has allowed the identification of the cold-responsive genes in upstream and downstream of *CBF*, and has provided evidence for the existence of a functional *CBF* cold response pathway [9–11]. This suggests that *CBF* genes act as key factors in regulating the cold-resistance process. Secondly, the prominent and conserved function of *CBF* in plants is argued through transgenic approaches. Overexpression of *CBF* genes from cold-tolerant or cold-sensitive plants is sufficient to induce constitutive expression of *CBF*-target genes and enhances cold tolerance in transgenic plants, no matter which are cold-tolerant or cold-sensitive species [12–15]. Therefore, *CBF* genes are important, evolutionarily conserved, components of the cold-resistance process in diverse plant species [16,17].

There is no doubt that *CBF* is vital in plants, but how important it is in fruits, is not well known. In a previous study, we discussed the function of the *CBF* gene in postharvest fruit [6]. We detected the expression of *LeCBF1* gene, which is functional in the cold response of tomato seedlings [7] and in postharvest fruits in cold storage (2 °C). We also compared the expression level in tomato fruits from two cultivars differing in their cold tolerances. The results indicated that in fruit, the gene swiftly responded to cold and its expression level was positively correlated with cold tolerance of the cultivars. Thus the *CBF* gene has the potential to be used

Abbreviations: *CBF*, C-repeat/dehydration-responsive element binding factor; COR, cold-related; Ct, threshold constant; *LeCBF1*, *Lycopersicon esculentum* putative transcriptional activator *CBF1*; QRT-PCR, quantitative (real-time) RT-PCR; RT-PCR, reverse transcription PCR; 1-MCP, 1-methylcyclopropene

* Corresponding author. Fax: +86 10 62737620.
E-mail address: pingshen@cau.edu.cn (J. Sheng).
[1] These authors contributed to this work equally.

0014-5793/$36.00
doi:10.1016/j.febslet.2009.09.029

as an indicator in cultivar selection and in chilling injury inspection. However, if we hope to establish method of evaluating tomato fruits' properties that is dependent on *LeCBF1* detection, we must know its gene expression pattern to determine the correct detection time and to identify major regulatory factors that might affect the utility of the method. In the present study, we determined the *LeCBF1* expression pattern in postharvest tomato fruits from all maturity stages and analyzed regulatory factors of its gene expression. The results provided us with the basic information necessary to establish the method of tomato condition evaluation.

2. Materials and methods

2.1. Plant material and treatment

Tomato fruits (*Solanum lycopersicum* cv. Lichun), free from blemishes or disease, were harvested at the mature green stage from a greenhouse in the China Agricultural University in June 2007. All fruits were selected for uniformity of shape, color (green), and size. The calyxes of the fruits were removed. Lichun fruits were randomly divided into three replicates in different baskets and stored at (2 ± 1) °C with 80–90% RH (relativity humidity) in cold storage for up to 3 weeks. The mesocarp from the fruit equator area was cut into small pieces, frozen in liquid N_2, and stored at −80 °C for enzyme and gene assays. For ethylene and 1-MCP treatments, two groups of fruits were dipped into 0.01% aqueous solution of ethephon for 10 min and treated with 500 nL L^{-1} 1-MCP (1-methylcyclopropene) at room temperature for 12 h, respectively. 1-MCP is a competitive inhibitor of ethylene binding that can block ethylene perception in plants.

Seedlings were grown in a growth chamber for 30 days with a 14 h light period under 4400 Lux light intensity at 28 °C and a 10 h dark period at 20 °C. For the cold stress treatment, uniformly developed 7-week seedlings were transferred to 4 °C at the fourth hour during the light period. New leaves were harvested directly into liquid N_2 and store at −80 °C for later use.

2.2. RT-PCR

Total RNAs were obtained by Trizol regent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was carried out using 2 μg total RNA treated with RNase-free DNase I (Promega, Madison, WI, USA) and AMV reverse transcriptase (Promega). The volume of each cDNA pool was adjusted to give the same exponential phase PCR signal strength according to the expression level of the tomato *Ubi3* gene (accession no. X58253) as an endogenous control [18]. The analysis of gene expression level was based on the band's intensity on an ethidium–bromide-stained gel.

2.3. Quantitative (real-time) RT-PCR

Quantitative RT-PCR was performed on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) [19]. For each reaction 20 ng of cDNA were used with SYBR GREENI MasterMix (Toyobo, Osaka, Japan). All experiments were run in triplicate with different cDNAs synthesized from three biological replicates. To determine relative fold differences for each sample, the threshold constant (Ct) value was normalized to the Ct value for *Ubi3*, and was calculated relative to a calibrator using the formula $2^{-\Delta\Delta Ct}$.

2.4. Measurement of chilling injury index

Chilling injury (CI) of fruits was evaluated at 20 °C for 3 days after 5-, 10-, 15-, and 20-days in cold-storage. The fruits were returned to ambient temperature (20 °C) for development of CI symptoms. Symptoms were manifested as surface pitting according to the method of Ding [20]. The severity of the symptoms was assessed visually using a four-stage scale: 0 = no pitting; 1 = pitting covering <25% of the fruit surface; 2 = pitting covering <50%, but >25% of surface; 3 = pitting covering <75%, but >50% of surface, and 4 = pitting covering >75% of surface. The average extent of cold damage was expressed as a CI index, which was calculated using the following formula: CI index (%) = $\{\sum[(\text{CI level}) \times (\text{Number of fruit at the CI level})]/(\text{Total number of fruits}) \times 4\} \times 100$.

2.5. Measurement of ion leakage

Ion leakage was measured immediately after 0-, 5-, 10-, 15-, and 20-days in cold storage (2 °C). The measurement method was that of Jiang with modifications [21]. Cylinders (3 mm thick) of mesocarp tissue were excised with a 1 cm diameter stainless steel cork borer from the equator part of four fruits. Disks were put into aqueous 0.1 M mannitol and shaken at 100 cycles per min for 2 h. The conductivity of the solution (L1) was measured with a conductivity meter (DDS-11A, Shanghai Leici Instrument Inc., Shanghai, China). Solutions were boiled for 10 min and then cooled to 20 °C. The conductivity of killed tissues (L2) was measured. Ion leakage was calculated as the ratio of L1 to L2.

2.6. Measurement of malondialdehyde content

Content of malondialdehyde (MDA) was measured immediately after 0-, 5-, 10-, 15-, and 20-days of cold storage (2 °C) using the thiobarbituric acid method described by Ding with modifications [22]. Absorbance at 532 nm was recorded and corrected for nonspecific absorbance at 600 nm. The amount of MDA was calculated from the extinction coefficient of 0.0155 μmol l^{-1} and expressed as μmol g^{-1}, where one unit was defined as 1 μmol MDA per g of pulp.

2.7. Measurement of proline content

The proline content was measured at the same time as the MDA measurement, using the acid ninhydrin method described by Bates with modifications [23]. Proline in tissues was extracted using 3% sulfosalicylic acid at 100 °C for 10 min with shaking. The extract was mixed with an equal volume of glacial acetic acid and acid ninhydrin reagent, and boiled for 30 min. After cooling, the reaction mix was partitioned against toluene, and the absorbance of the organic phase was recorded at 520 nm. The resulting values were compared with a standard curve constructed using known amounts of proline (Sigma, St Louis, MO, USA).

2.8. Statistical analysis and experiment replicates

A completely randomized design with three replicates per cultivar, where each basket constituted a replicate, was performed. Data were analyzed for significant differences by one-way analysis of variance (ANOVA) using the statistical software SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Significant effects were determined using LSD multiple comparison procedure at the 5% level and 1% level were considered significant and extremely significant, respectively.

3. Results

3.1. Double-peak expression pattern of LeCBF1 in mature green fruits

The *LeCBF1* expression pattern in mature green fruit was analyzed and compared with that of seedlings by QRT-PCR. As shown