Biochemical and Biophysical Research Communications 442 (2013) 116–121

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Capsicum annuum homeobox 1 (CaHB1) is a nuclear factor that has roles in plant development, salt tolerance, and pathogen defense

Sang-Keun Oh [a,1], Joonseon Yoon [a,1], Gyung Ja Choi [b], Hyun A Jang [c], Suk-Yoon Kwon [c], Doil Choi [a,*]

[a] Department of Plant Science, College of Agriculture and Life Sciences, Seoul National University, Seou1 151-742, Republic of Korea
[b] Screening Division, Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea
[c] Korea Research Institute of Bioscience and Biotechnology, Yusung, Daejeon 305-600, Republic of Korea

ARTICLE INFO

Article history:
Received 30 October 2013
Available online 14 November 2013

Keywords:
Homeodomain-leucine zipper
CaHB1
Phytophthora infestans
Plant defense
Salt tolerance

ABSTRACT

Homeodomain-leucine zipper (HD-Zip) family proteins are unique to plants, but little is known about their role in defense responses. CaHB1 is a nuclear factor in peppers, belonging to subfamily II of HD-Zip proteins. Here, we determined the role of *CaHB1* in the defense response. *CaHB1* expression was induced when pepper plants were challenged with *Phytophthora capsici*, a plant pathogen to which peppers are susceptible, or environmental stresses such as drought and salt stimuli. *CaHB1* was also highly expressed in pepper leaves following application of SA, whereas ethephon and MeJA had a moderate effect. To further investigate the function of *CaHB1* in plants, we performed gain-of-function study by overexpression of *CaHB1* in tomato. *CaHB1*-transgenic tomatoes showed significant growth enhancement including increased leaf thickness and enlarged cell size (1.8-fold larger than control plants). Microscopic analysis revealed that leaves from *CaHB1*-transgenic plants had thicker cell walls and cuticle layers than those from controls. Moreover, *CaHB1*-transgenic plants displayed enhanced resistance against *Phytophthora infestans* and increased tolerance to salt stress. Additionally, RT-PCR analysis of *CaHB1*-transgenic tomatoes revealed constitutive up-regulation of multiple genes involved in plant defense and osmotic stress. Therefore, our findings suggest roles for *CaHB1* in development, salt stress, and pathogen defense.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

The homeodomain leucine zipper (HD-Zip) family of proteins in plants is a group of transcription factors that regulate development in response to environmental or external stresses [1,2]. The family is classified into four subfamilies (HD-Zip I-IV) by DNA-binding, conserved motif, and function [2]. The genes in subfamily I and II are involved in adaptation to external stimuli such as drought and salinity [3,4]. The H52 protein in tomato, a member of the HD-Zip family, limits cell death during pathogen infection [5]. Silencing of H52 in tomatoes resulted in cell death, which spread to uninfected neighboring plant parts during infection [5].

Osmotic treatment induced the expression of a number of HD-Zip family genes, namely *Arabidopsis thaliana HB6* (*AtHB6*), *Barssica napus HB6* (*BnHB6*), and *Oriza sativa hox22* (*Oshox22*) [6–8]. These genes were up-regulated by various environment stresses and by externally applied abscisic acid (ABA) or salicylic acid (SA) [6–8]. Zhang et al. [8] demonstrated that overexpression of *Oshox22* reduced tolerance to salt stress at the rice seedling stage through an ABA-mediated signaling pathway. However, there is no available information about the *Homeobox* (*HB*) gene-mediated defense response to pathogen attack.

Recently, we described the *NbHB1* gene, which encodes an HD-Zip protein from *Nicotiana benthamiana* [9]. Using transient or virus-induced gene silencing in *N. benthamiana*, we showed that *NbHB1* positively regulated pathogen-induced cell death. In our studies, expression of *NbHB1* following inoculation with pathogens and various other treatments was enhanced [9]. Ectopic expression of *NbHB1* accelerated cell death following abiotic stresses or bacterial pathogen inoculation [9]. According to protein databases, the NbHB1 protein belongs to subfamily II with an *N*-terminal variable region and *C*-terminal conserved CPSCE motif [1,9]. We also identified *Capsicum annuum homeobox 1* (*CaHB1*), an HD-Zip II family gene, in pepper plants infected with a bacterial pathogen [9]. However, the role of *CaHB1* in disease resistance is unknown.

Here, we describe the role of *CaHB1* in plant defense against pathogens using gain-of-function studies in transgenic tomato (*Solanum lycopersicon* L. cv. 'MicroTom') plants overexpressing

* Corresponding author. Address: Department of Plant Science, Plant Genomics and Breeding Institute, Institute of Agricultural Biotechnology, Seoul National University, Seou1 151-742, Republic of Korea.

E-mail address: doil@snu.ac.kr (D. Choi).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.11.019

CaHB1. We believe these results will considerably enhance understanding of the role of HD-Zip genes in defense against pathogens and environmental stresses.

2. Materials and methods

2.1. Plant materials

For tomato (*S. lycopersicon* L. cv. 'MicroTom') transformation, seeds were surface-sterilized in 1% (v/v) NaOCl, followed by wash with sterilized distilled water. Seeds were then germinated on MS agar medium and kept in a plant growth chamber under a 16-h photoperiod at 25 °C for 2 weeks before being used for transformation [10]. Tomato and pepper plants (cv. Bugang) were grown in pots and maintained under a 16-h photoperiod at 25 °C.

2.2. Agrobacterium-mediated transformation of tomato

A method for generation of transgenic plants was used as described by Oh et al. [10]. Briefly, cotyledons from 2-week-old tomato seedlings germinated on MS medium were used for co-cultivation with Agrobacterium to generate transgenic plants [10]. Kanamycin-selected transgenic plants were grown in a greenhouse, and then transgenic plants were further selected by measuring the expression of the nopaline synthase terminator by PCR with a primer set (Table S1).

2.3. Evaluation of CaHB1-transgenic tomato resistance to Phytophthora infestans

Four-week-old T3-tomato plants were inoculated with the zoospores of *P. infestans*. *P. infestans* infection of tomato plants were performed using zoospores inoculations as described by Oh et al. [10]. Disease symptoms appeared within 2 to 3-DAI from 5–6 independent infected plants. The index of *Phytophthora* infection was determined visually based on the necrotic leaf area [10].

2.4. Chlorophyll content analysis

For the leaf disk assay, tomato leaf disks with a diameter of 10 mm ($n = 5$) were prepared from tomato leaves of identical development stage of both vector-control and transgenic plants and floated on solutions of different NaCl concentrations for 3-days. After treatment with NaCl (0.5 and 1.0 M), chlorophyll was extracted from transgenic or control only transgenic-tomato leaves according to the method of Hu et al. [11], and then the chlorophyll content was calculated by the method of Lichtenthaler [12]. The photographs were taken at 3 days, and the experiments were repeated at least twice.

2.5. Microscopic analysis

Microscopic analysis was carried out following the methods as described by Sarowar et al. [13]. Leaves of one-month-old control or *CaHB1*-transgenic plants were observed under a light microscope and a transmission electron microscope (TEM).

2.6. Chemical treatment and pathogen inoculation

To determine the expression of the *CaHB1* transcripts after treatment of 5 mM SA, 5 mM ethephone, 100 μM MeJA, or 100 μM ABA, leaves of pepper plants were sprayed with chemicals as described in Oh et al. [10].

2.7. Subcellular localization of CaHB1 protein

A construct, CaHB1-smGFP under the control of CaMV-35S promoter, was made for CaHB1 expression in *N. benthamiana* protoplast. The fusion constructs were introduced into *N. benthamiana* protoplasts prepared from young leaves by the polyethylene glycol-mediated transformation Oh et al. [10]. Expression of the fusion constructs was observed at 40 h after transformation using a confocal laser scanning microscope (Carl Zeiss LSM 510), and the image was captured with a cooled charge-coupled device camera. The filter sets were used as described in Oh et al. [10].

2.8. RT-PCR analysis

Total RNA samples were extracted from pepper using TRI reagent according to the manufacturer's instructions (Invitrogen). RT-PCR was performed to detect the endogenous levels of several genes using the primer sets listed in Supplemental Table S1. The expression of the PiEF1a gene was controlled with a primer pair specific for the constitutively expressed tomato *Actin* gene (Table S1).

3. Results

3.1. Expression and cellular localization of CaHB1

We isolated *CaHB1* using genome-wide screening, which identifies genes up-regulated during pepper-bacterial pathogen interaction [9,14]. This gene's (NCBI accession No. EU998972) open reading frame encodes 272 amino acid residues (Fig. S1) and, according to protein databases, contains an N-terminal variable region, an HD-Zip domain, and a C-terminal conserved CPSCE motif. Therefore, it belongs to subfamily II of the HD-Zip family [1,9]. The overall structure of CaHB1 is similar to other HD-Zip family plant proteins, especially NbHB1, with which it shares 84% amino acid identity (Fig. S1).

The expression of *CaHB1* was determined in different tissues of the pepper plant. High levels of *CaHB1* transcripts were present in flowers and stems, while levels were low in leaves and roots (Fig. 1A). *CaHB1* was highly expressed during development in pepper leaves, indicting *CaHB1* regulates senescence (Fig. 1B).

HD-Zip proteins are transcription factors; therefore, we determined whether CaHB1 is present in the nucleus. Although we did not find putative nuclear localization sequences in CaHB1 with the PSORT II program (http://psort.nibb.ac.jp), other evidence indicates that it is localized in the nucleus. CaHB1 was tagged at the C-terminal with soluble modified green fluorescent protein (smGFP) and constitutively expressed using the CaMV-35S promoter (Fig. 1C). The fused construct was transformed into protoplasts of *N. benthamiana*, and GFP florescence was localized in the nucleus (Fig. 1C). In contrast, with the 35S-smGFP control, GFP fluorescence was observed throughout the cytoplasm and the nucleus. Like other HD-Zip proteins, CaHB1 appears to be a nuclear transcription factor.

3.2. Effect of biotic and abiotic stresses on CaHB1 expression

To understand the function of CaHB1 in stress, the expression levels of *CaHB1* were monitored in pepper plants treated with biotic and abiotic stress. *CaHB1* expression was strongly induced when pepper leaves were inoculated with the pepper pathogen, *Phytophthora capsici*. Likewise, *CaHB1* transcripts increased remarkably after inoculation, before disease symptoms were visible, and remained elevated until 72 h after inoculation (Fig. 1D). *CaPR-1* transcript, a marker gene, was detected 48 h after inoculation and remained high for 72 h (Fig. 1D).

Download English Version:

https://daneshyari.com/en/article/8297604

Download Persian Version:

https://daneshyari.com/article/8297604

Daneshyari.com