Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Plant Physiology and Biochemistry 46 (2008) 205−211
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Research article

Increased freezing tolerance through up-regulation of downstream genes via the wheat *CBF* gene in transgenic tobacco

Shigeo Takumi*, Chisa Shimamura, Fuminori Kobayashi[1]

Laboratory of Plant Genetics, Graduate School of Agrobiological Science, Kobe University, Rokkodai-cho 1-1, Nada-ku, Kobe 657-8501, Japan

Received 29 May 2007
Available online 26 October 2007

Abstract

The wheat (*Triticum aestivum* L.) *CBF* gene family is assumed to play important roles in development of low-temperature and freezing tolerance through activation of the downstream *Cor*/*Lea* genes. However, no direct evidence shows association of the wheat *CBF* genes with stress tolerance or any interaction between wheat CBF transcription factors and *Cor*/*Lea* gene activation. Here, we introduced *Wcbf2*, one of the wheat *CBF* genes, into the tobacco (*Nicotiana tabacum* L.) genome. Expression of *Wcbf2* significantly increased the level of freezing tolerance in the transgenic tobacco plants without phenotypic retardation, and altered the expression patterns of tobacco genes, including cold-responsive genes. A transgenic tobacco plant expressing *Wcbf2* was crossed to other transgenic plants expressing a *GUS* reporter gene under control of the wheat *Cor*/*Lea* gene promoter. Analysis of the F_1 plants showed that the WCBF2 protein positively regulated at least the expression of *Wdhn13* and *Wrab17*. These results strongly indicate that WCBF2 functions as a transcription factor in the development of freezing tolerance in common wheat.
© 2007 Elsevier Masson SAS. All rights reserved.

Keywords: CBF transcription factor; *Cor*/*Lea* genes; Differential display; Freezing tolerance; Transgenic plant; *Triticum aestivum* L

1. Introduction

Cold acclimation is an adaptive process for acquiring freezing tolerance in higher plants. In the cold-acclimation process, a large number of low-temperature responsive genes are transcriptionally activated, and the accumulated proteins and metabolites lead to the protection of cell structures and functions from freezing damage [33]. The low-temperature responsive genes are called *Cor* (cold-responsive)/*Lea* (late-embryogenesis-abundant) genes. A functional cis-acting element, i.e., the CCGAC core motif known as a CRT (C-repeat)/DRE (dehydration-responsive element) sequence, was proven to play a pivotal role in the promoter function of *COR15A*/*RD29A* genes in *Arabidopsis* [3,35]. A family of transcription factors called CRT-binding factors (CBFs) or DRE-binding proteins (DREBs) regulates *Cor*/*Lea* gene expression through binding to the CRT/DRE cis elements. These transcription factors contain a DNA binding domain found in the ethylene-responsive element binding protein/APETALA2 (EREBP/AP2) family [16,28]. The CBF/DREB1 transcription factors are key regulators of cold signal transduction in various plant species [6,17,26,33]. Overexpression of *Arabidopsis CBF1* not only leads to strong expression of *Cor*/*Lea* genes, but also improves freezing tolerance [8,10,16].

In common wheat, many low temperature-responsive *Cor*/*Lea* genes have been characterized [5,11,21,31,32]. Wheat *CBF* homologs such as *TaCBF*, *TaDREB1* and *Wcbf2* have also been isolated and characterized [7,14,23]. Recently, a cluster of 11 *CBF* genes in einkorn wheat was located to the *Fr-A^{m}2* locus, which maps as a quantitative trait locus (QTL) for freezing tolerance on chromosome $5A^m$ [18,34]. The *Fr-A^{m}2* locus controls *Cor*/*Lea* gene expression and freezing tolerance [34]. Another QTL for freezing tolerance, *Fr-1*,

Abbreviations: CaMV, cauliflower mosaic virus; COR, cold-responsive; CBF, CRT-binding factor; CRT, C-repeat; DRE, dehydration-responsive element; GUS, β-glucuronidase; LEA, late-embryogenesis-abundant; MS, Murashige-Skoog; QTL, quantitative trait locus; RAPD, randomly amplified polymorphic DNA.

* Corresponding author. Tel.: +81 78 803 5860; fax: +81 78 803 5859.
E-mail address: takumi@kobe-u.ac.jp (S. Takumi).
[1] Present address: Plant Genome Research Unit, National Institute of Agrobiological Sciences, 2-1-2 Kan-non-dai, Tsukuba 305-8602, Japan.

0981-9428/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.plaphy.2007.10.019

was also assigned to the homoeologous group 5 chromosomes [4,29], and it was strongly suggested that the *Fr-1* locus controls development of freezing tolerance and *Cor/Lea* gene expression through *CBF* transcriptional activation [12]. Therefore, *Fr-1* and *Fr-2* loci regulate wheat freezing tolerance through *Cor/Lea* expression. However, there is no direct evidence for trans-activation of *Cor/Lea* expression via the *CBF* genes in wheat. Our previous study showed that a reporter gene under control of the promoter sequences of two wheat *Cor/Lea* genes, *Wcor15* and *Wdhn13*, seemed to be slightly activated by co-transformed *Wcbf2* in wheat cultured cells, although the results were not statistically significant [14].

In our previous study, overexpression of *Wcor15*, a member of the wheat *Cor/Lea* gene family, improved freezing tolerance in transgenic tobacco plants, but the increase in freezing tolerance was observed only under limited conditions [24]. Restricted but significantly improved levels of freezing tolerance were also reported in transgenic *Arabidopsis* plants expressing the *Cor15a* and *Wcs19* genes [2,20]. Therefore, overexpression of the CBF transcription factor might lead to higher levels of freezing tolerance than that of individual COR/LEA proteins. In fact, expression of an *Arabidopsis CBF* gene greatly increased transcript accumulation levels of many *Cor/Lea* genes and the freezing tolerance level in transgenic plants [8,10,16,22].

The aim of the present study was to clarify the in vivo interaction between *Wcbf2* and *Cor/Lea* genes. Here, we report production of transgenic tobacco plants expressing *Wcbf2* and/or *Cor/Lea* promoter-containing reporter genes, trans-activation of the reporter gene and alteration of freezing tolerance.

2. Methods

2.1. Vector construction and tobacco transformation

Wcbf2 cDNA clones were introduced into the *Xba*I/*Sac*I site of pBI121 (Clontech) to produce a *CaMV35S::Wcbf2* construct. The construct was introduced into leaf discs of tobacco (*Nicotiana tabacum* L. 'Petite Havana') using *Agrobacterium tumefaciens* LBA4404. Transformants were selected on Murashige-Skoog (MS) medium [19] containing 0.1 mg L^{-1} alpha-naphthalene acetic acid, 1.0 mg L^{-1} 6-benzyl aminopurine and 250 mg L^{-1} kanamycin, and regenerated on hormone-free MS medium containing 50 mg L^{-1} kanamycin.

For Southern blot analysis, total DNA extracted from tobacco leaves was digested with a restriction enzyme HindIII. The digested DNA was fractionated by electrophoresis through an 0.8% agarose gel, transferred to Hybond N^{+} nylon membrane (GE Healthcare, Piscataway, NJ, USA) and hybridized with ^{32}P-labeled *Wcbf2* cDNA as a probe. Probe labeling, hybridization, washing and autoradiography were performed according to Kume et al. [14].

2.2. Bioassay for freezing tolerance

Two types of transgenic tobacco plants were used for determination of freezing tolerance. The *CaMV35S::Wcbf2* transgenic plants were produced in this study. Another transgenic tobacco, *35S::Wcor15*, was previously reported [24]. Two-week-old seedlings of transgenic tobacco plants were grown on the MS medium in a controlled-climate cabinet at 25 °C with a 16 h photoperiod at a light intensity of 110–120 μm photons m^{-2} s^{-1} provided by cool white fluorescence lamps. The seedlings were treated with or without cold acclimation at 4 °C for 3 days and then frozen at −15 °C for 1 or 2 h in the dark. The frozen seedlings were thawed overnight at 4 °C and transferred back to normal temperature conditions (25 °C). On the 14th day after transfer, survival of seedlings was recorded. The whole experiment was repeated three times and the data were statistically analyzed by Student's *t*-test between the wild-type plant and transgenic lines.

2.3. cDNA differential display with RAPD primers

Low temperature treatment for 3 days was given by transferring 14-day-old seedlings from normal temperature conditions to cold acclimation conditions (4 °C) for 5 days. Total RNA was extracted by guanidine thiocyanate from cold-acclimated and non-acclimated tobacco leaves. First-strand cDNA was synthesized from DNase I-treated RNA samples with oligo-dT primers using ReverTra Ace® (Toyobo, Osaka, Japan). A differential display method [15] was performed using the first-strand cDNA as template. A total of 50 random 10-mer primer combinations (Operon Technologies, Inc., CA, USA) were used for identification of transcripts abundant in transgenic tobacco plants. PCR amplification was initiated at 95 °C for 1 min, followed by 35 cycles of 94 °C for 1 min, 40 °C for 1 min, and 72 °C for 2 min, and terminated at 72 °C for 1 min. After amplification, the resulting fragments were separated on a 1.5% agarose gel. These fragments were cloned into pGEM-T Easy vector (Promega, WI, USA) and sequenced. Expression of these clones was studied by RT-PCR using the following two gene-specific primer sets: 5′-ATGTCGTCGCCGTCGCGCCG-3′ and 5′-CTCCGGGGATGTCCACGGGG-3′ for the R15Y15 fragment and 5′-GAGATCTGAGTAGGTGA-3′ and 5′-CTAG CAATCCATCCATC-3′ for the U5V5-1 fragment. The annealing temperatures for the RT-PCR amplification were 55 °C and 45 °C for the R15Y15 and U5V5-1 fragments, respectively. Thirty-five cycles of PCR were performed and the amplified products were separated by electrophoresis through a 1.5% agarose gel and stained with ethidium bromide.

2.4. Crossing of transgenic tobacco plants and GUS assay

The CaMV35S promoter of pBI121 was exchanged for the 5′ upstream sequence of wheat *Cor/Lea*, i.e., *Wcor15*, *Wdhn13*, *Wrab17* or *Wrab19*, and four chimeric *GUS* genes were constructed using these sequences, then introduced into the tobacco genome by the *Agrobacterium*-infection method [31]; these were named, respectively, *Wcor15 pro::GUS*, *Wdhn13 pro::GUS*, *Wrab17 pro::GUS* and *Wrab19 pro::GUS*. *CaMV35S::Wcbf2* transformants were used as pollen parent in crosses with transgenic tobacco plants expressing a chimeric GUS gene under

Download English Version:

https://daneshyari.com/en/article/2015405

Download Persian Version:

https://daneshyari.com/article/2015405

Daneshyari.com