GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Characterization of *FeDREB1* promoter involved in cold- and drought-inducible expression from common buckwheat (*Fagopyrum esculentum*)

Z.W. Fang[1,2]*, X.Y. Xu[1]*, J.F. Gao[1], P.K. Wang[1], Z.X. Liu[2] and B.L. Feng[1]

[1]State Key Laboratory of Crop Stress Biology for Arid Areas/
College of Agronomy, Northwest A&F University, Yangling, Shanxi, China
[2]Institute of Crop Genetics and Breeding/College of Agriculture,
Yangtze University, Jingzhou, Hubei, China

*These authors contributed equally to this study.
Corresponding author: B.L. Feng
E-mail: fengbaili@nwsuaf.edu.cn

Genet. Mol. Res. 14 (3): 7990-8000 (2015)
Received October 16, 2014
Accepted February 19, 2015
Published July 17, 2015
DOI http://dx.doi.org/10.4238/2015.July.17.7

ABSTRACT. C-repeat-binding factor (CBF)/dehydration-responsive element (DREB) transcription factors play key roles in plant stress responses. However, little information is available on the regulation of CBF/DREB expression. In this study, we isolated and characterized the *FeDREB1* promoter sequence from the common buckwheat accession Xinong 9976. To identify the upstream region of the *FeDREB1* gene required for promoter activity, we constructed a series of *FeDREB1* promoter deletion derivatives. Each deletion construct was analyzed through *Agrobacterium*-mediated transient transformation in tobacco leaves treated with 4°C cold or drought stress. Promoter-beta-glucuronidase fusion assays revealed that the *pCD1* (-270 bp) deletion in the upstream region of *FeDREB1* could activate expression of the *GUS* gene at 4°C. The *pCD1* (-270 bp), *pCD2* (-530 bp), and *pCD3*

(-904 bp) deletion induced low-level *GUS* expression under drought stress. However, the *pCD4* (-1278 bp) deletion clearly activated *GUS* gene expression. Our results suggest that sections *pCD1* (-270 bp) and *pCD4* (-1278 bp) in the *FeDREB1* gene promoter are new sources of induced promoters for adversity-resistance breeding in plant genetic engineering.

Key words: Cold and drought stress; *Fagopyrum esculentum*; *FeDREB1* promoter

INTRODUCTION

Abiotic stresses such as low temperature and drought can have serious impacts on the growth and development of plants because of their sessile nature (Agarwal et al., 2006b). Plants have evolved a variety of responsive mechanisms to cope with negative environments on the molecular, cellular, and physiological levels (Ramanjulu and Bartels, 2002; Dubouzet et al., 2003; Rabbani et al., 2003). Within these responsive approaches, C-repeat binding factor/dehydration responsive element binding factor (CBF/DREB) can specially combine with the *cis*-acting element C-repeat/dehydration-responsive element, which is located in the downstream gene promoter, and then activate the expression of relevant genes to enhance the stress resistance of plants (Gilmour et al., 1998; Shinozaki and Yamaguchi-Shinozaki, 2000; Cook et al., 2004; Yamaguchi-Shinozaki and Shinozaki, 2006; Dietz et al., 2010). Numerous CBF/DREB homologous genes have been cloned and identified in model species such as *Arabidopsis* (Liu et al., 1998), and in major crops such as rice (Tian et al., 2005; Ito et al., 2006), wheat (Shen et al., 2003), maize (Kizis, 2002; Qin et al., 2004), soybean (Chen et al., 2009), and cotton (Qiao et al., 2008). Therefore, it is necessary to investigate the signal transduction pathway and expression regulation mechanism of the *CBF/DREB* gene family because of its increasingly important value in stress-resistance applications in plants. Previous studies have mainly focused on the expression regulation mechanism of CBF/DREB in the response to cold stress. Shinwari et al. (1998) found that the *cis*-acting element responding to low temperature in the promoter of the *CBF/DREB* gene of *Arabidopsis* may play a pivotal role in gene expression. A transcription factor gene, inducer of CBF expression 1 (*ICE1*), was identified through map-based cloning of *Arabidopsis*. *ICE1* encodes an MYC-like basic helix-loop-helix (bHLH) protein that binds specifically to MYC sites in the DREB1A/CBF3 promoter region and increases the expression of *DREB1A/CBF3* under cold stress (Chinnusamy et al., 2003). However, overexpression of the dominant gene *ice1*, which corresponds to *ICE1*, did not influence the expression of CBF2/DREB, suggesting that different members in the *CBF/DREB1* gene family have distinct expression regulation mechanisms (Chinnusamy et al., 2003). In addition to MYC-like bHLH proteins, other proteins affecting the expression of *DREB1/CBF*-like genes have also been identified. Gong et al. (2002) found that the *LOS4* gene positively affected the expression of CBF/DREB; *LOS4* codes for a DEAD-box RNA helicase located in the cell nucleus, whose over-expression can increase the accumulation of CBF/DREB1 in plants. In contrast, a negative feedback regulation gene within CBF/DREB1 has been identified. Lee et al. (2001) isolated an E3 ligase *HOS1* gene that is responsible for the ubiquitination of the CBF/DREB regulation protein and protein degradation, and Xiong et al. (2002) identified the *FRY2* gene, which regulates pre-mRNA processing during transcription. Their

results demonstrated that at low temperatures, the *HOS1* and *FRY2* genes inhibited the expression of CBF/DREB1 in *Arabidopsis* (Lee et al., 2001; Xiong et al., 2002). MYB15 is an R2R3-MYB family protein, which can combine with the MYB-binding site at the *CBF/DREB* upstream promoter and repress the expression of *CBF/DREB* genes in *Arabidopsis* (Agarwal et al., 2006a). In addition, some *CBF/DREB* promoters found to be responsive to cold or heat stress have been identified. For example, a 125-base pair (bp) region of the *DREB1C* promoter was found to be sufficient for driving cold-induced transcription, and 2 segments within the promoter, designated *ICEr1* and inducer of *CBF* expression region 1 and 2, contribute to the cold responses as well (Zarka et al., 2003). A 394-bp (-664 to -1058) region of the *GmDREB3* promoter was found to be capable of driving beta-glucuronidase (*GUS*) expression under cold stress (Chen et al., 2009). GUS analysis of a series of 5'-deletions in the *DREB2C* promoter revealed that the proximal region of the *DREB2C* promoter between positions -34 and -204 bp were capable of gene induction under heat stress (Chen et al., 2012). However, the regulatory mechanisms of most *CBF/DREB* genes remain unclear, likely because the promoters have not been widely examined. To isolate additional *CBF/DREB* promoters from plants while simultaneously identifying the function of the relevant responsive promoter would improve our understanding of the expression regulation mechanism of *CBF*/*DREB*. The *FeDREB1* gene (Gene No.: JN600617) was isolated from common buckwheat (*Fagopyrum esculentum*). Expression of the *FeDREB1* gene was absent under normal conditions and was up-regulated under cold or drought stress in common buckwheat (data not shown). Hence, we speculated that the *FeDREB1* promoter was environmentally induced. Thus, we investigated the regulatory mechanism of *FeDREB1* in common buckwheat. We isolated and conducted functional characterization of the promoter regions of the *FeDREB1* gene in the common buckwheat accession Xinong 9976. Understanding this novel promoter will benefit future studies aimed at understanding gene expression regulation.

MATERIAL AND METHODS

Isolation and analysis of *FeDREB1* promoter

Buckwheat genomic DNA was isolated from young Xinong 9976 leaves grown under a 16-h light/8-h dark photoperiod at 25°C for 15 days. The 5'-flanking region of *FeDREB1* was segregated from the buckwheat genomic DNA using the Genome Walking kit (TaKaRa, Shiga, Japan) following the manufacturer protocol and using gene-specific primers. A primary polymerase chain reaction (PCR) to amplify the 5'-regulatory region was performed using degenerate primers (AP1, AP2, AP3, and AP4) and the gene-specific primer p-FeDREB1-Sp1 (5'-GAA TCA GCA AAA TTG AGA CAC GCG-3'). Primary PCR products diluted 10X were then amplified using degenerate primers (AP1, AP2, AP3, and AP4) and the nested primer p-FeDREB1-Sp2 (5'-GTT CGC ATG CCG GAT CTC ACA TAC-3'). The second PCR products diluted 10X were then amplified using degenerate primers (AP1, AP2, AP3, and AP4) and the nested primer p-FeDREB1-Sp3 (5'-GCT TTA GTC CTC CTG CTG AGA AAG-3'). PCR amplifications were performed as described by the manufacturer. The nested PCR products were purified on a 1.5% agarose gel, and then cloned into the pMD-19-T vector (TaKaRa) and sequenced. The promoter sequence was analyzed by Plant CARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and subsequently analyzed with the PLACE (http://www.dna.affrc.go.jp/PLACE/) database (Higo et al., 1999; Lescot et al., 2002).

Construction of reporter plasmids

To generate the reporter plasmids for the *Agrobacterium*-mediated transient expression system, the *GUS* gene from pCAMBIA1391 was cloned into the pCAMBIA0390 vector to generate *pC0390::GUS* and used as a negative control (Xu et al., 2010). The CaMV 35S promoter from pBI121 was introduced into pC0390GUS to generate the positive control *pC35S::GUS* construct. To prepare the constructs containing various promoter fragments, PCR products were amplified from the cloned *FeDREB1* promoter using primers that incorporated *Hin*dIII and *Bam*HI sites at the 5' and 3' ends of the promoter fragments, respectively. A series of nested 5'-deletions of *FeDREB1* promoter fragments were generated using PCR amplification from the cloned *FeDREB1* promoter. The forward primers pCD1-F (5'-CCC AAG CTT ATA GAC CTA GTC AGG ATC A-3'), pCD2-F (5'-CCC AAG CTT CGT AGT TGA AGA TTA GTC-3'), pCD3-F (5'-CCC AAG CTT ATG ATC GGG TTG TGA TAC-3'), pCD4-F (5'-CCC AAG CTT AAG GTT CTT TGC AAG TC-3'), and pCD5-F (5'-CCC AAG CTT GCG TCA CAT TTA TTA TTT TA-3') were designed to correspond to the -270, -530, -904, -1278, and -1710 bp sequences of the *FeDREB1* promoter. The reverse primer, pCD-R (5'-GCG GAT CCG ATC GAT CCT GAA TAT AT-3'), was located at the 3'-end of the *FeDREB1* promoter. A *Hin*dIII restriction enzyme site (underlined sequences) was introduced at the 5'-end of each of the forward primers, and a *Bam*HI site (underlined sequence) was added at the 3'-end of the reverse primer. Each of the 5 promoter fragments was double-digested with *Hin*dIII/*Bam*HI and ligated into the *Hin*dIII/*Bam*HI site of the vector *pC0390::GUS*, immediately upstream of the ATG start codon for β-glucuronidase. Each deletion construct was introduced into *Agrobacterium tumefaciens* strain GV3101 via electroporation.

Agrobacterium-mediated transient assay

Agrobacterium-mediated transient assays were performed as described previously (Sparkes et al., 2006), with some modifications. A single colony of *Agrobacterium* strain GV3101 harboring the *FeDREB1* promoter deletion was inoculated into 20 mL Luria-Bertani (LB) liquid medium supplemented with 50 μg/mL rifampicin and 50 μg /mL kanamycin at 28°C for 2 days. The cultures were then grown in 50 mL fresh LB liquid medium at 28°C overnight. *Agrobacterium* cells were harvested after centrifugation at 5000 *g* for 10 min, re-suspended in infiltration solution [10 mM 4-morpholineethanesulfonic acid (MES), pH 5.7, 10 mM $MgCl_2$, and 100 μM acetosyringone], and adjusted to an optical density at 600 nm of 0.6 for infiltration into the fully expanded and young tobacco leaves. The infiltrated tobacco plants were maintained in a moist chamber with a 16-h light/8-h dark cycle at 25°C with 75% humidity for 24 h. For cold stress, the infiltrated tobacco plants were incubated at 4°C. For drought stress, the infiltrated tobacco plants were placed into the 15% polyethylene glycol 6000 for 24 h. The infiltrated tobacco plants under normal growth conditions were used as controls for cold and drought stress, respectively. After 24-h treatment under cold or drought treatment, tobacco leaves were collected and stored at -80°C until measurement of GUS activity. All experiments were repeated at least 3 times.

GUS activity assay and protein determination

Agrobacterium-infiltrated leaf samples from each treatment were collected for each

construct to determine GUS activity. Quantitative GUS assays of crude extracts were performed as described previously (Jefferson, 1987) using 4-methylumbelliferyl glucuronide (Sigma-Aldrich, St. Louis, MO, USA) as a substrate. GUS activity was determined using a Hitachi 850 Fluorescence spectrophotometer (Hitachi, Tokyo, Japan), and the total concentration of protein extracts from the samples tested was normalized by diluting the samples with extraction buffer as described previously (Bradford, 1976). GUS activity was expressed in nanomolar units of 4-methylumbelliferon (Sigma-Aldrich) generated per minute per milligram of soluble proteins. For histochemical staining, tobacco leaf tissues were immersed into GUS staining solution [10 mM Na_2EDTA, 100 mM NaH_2PO_4, 0.5 mM $K_4Fe(CN)_6 \cdot 3H_2O$, 0.1% TritonX-100, and 0.5 µg/mL 5-bromo-4-chloro-3-indolyl-b-D-glucuronic acid (X-Gluc, Sigma-Aldrich), pH 7.0] and incubated for 24 h at 37°C, followed by chlorophyll removal from the leaves by successive washes with 70% ethanol at 37°C. Data are mean values from 3 independent assays of tobacco leaf extracts and error bars represent the standard deviations of the replicates. A one-side paired Student *t*-test was performed to assess significant differences between control and treatment conditions using the statistical software package SigmaPlot 11.0 (Ashburn, VA, USA). Differences of $P < 0.01$ were considered significant.

RESULTS

Sequence analysis of the *FeDREB1* promoter

Using the genome-walking method, a 1710-bp *FeDREB1* promoter was obtained from the genome of the common buckwheat accession Xinong 9976. The *FeDREB1* promoter sequence was then analyzed using the databases of the PlantCARE and PLACE websites. The results showed that the *FeDREB1* promoter harbored numerous *cis*-acting elements, including 24 predicted TATA-boxes, 8 CAAT-boxes, 4 MYCCONSENSUSAT drought and low temperature-responding elements (CANNTG), 1 MYBCORE drought responding element (CNGTTR), 1 MYB1AT drought responding element (WAACCA), 1 ACGTATERD1 drought-responding element (ACGT), 1 LTRECOREATCOR15 low temperature-responding element (CCGAG), 1 TC-rich repeat adversity corresponding element (AGTTTCTTCA), 1 TCA1MOTIF salicylic acid-responding element (TCATCTTCTT), and 2 ABRE-responding elements (ACGTG) (Figure 1). Moreover, responsive elements such as ARFAT growth hormone (CCCAC), JERE MeJA (AGACCGCC), and 10 DOF protein-binding sites (AAAG) were involved, and MYB1LEPR defending-responsive elements such as (GTTAGTT) and WBOXATNPR1 disease-responding element (TTGAC) were found (Rushton et al., 1996) (Figure 1). Most predicted motifs were involved in the response to environmental stress, suggesting that the *FeDREB1* promoter plays an important role in abiotic response regulation.

Activation of the *FeDREB1* promoter under cold stress or drought stress

To identify the core functional region of the *FeDREB1* promoter involved in the response to cold stress, we generated a set of serial deletions of the *FeDREB1* promoter by PCR (Figure 2). The deletions, beginning at the locations -270, -530, -904, -1278, and -1710 bp, described relative to the *FeDREB1* transcription start site, were fused with the GUS reporter. Histochemical GUS assays showed that under control, low temperature, and drought circumstances, the wild-type and *pC0390::GUS* transformed plant expression vector (negative con-

trol) of the tobacco leaves were not stained by the X-Gluc solution, but tobacco leaves that had been transformed with the *pC35S::GUS* plant expression vector (positive control) were stained blue by X-Gluc solution under each treatment (Figures 3 and 4). Figure 3 shows that compared with the control, which grows under normal conditions, the *pCD1::GUS* transformed tobacco leaves were dyed blue by X-Gluc under 4°C cold stress, but the *pCD2::GUS*, *pCD3::GUS* and *pCD4::GUS* vectors were not stained, indicating that the *pCD1* (-270 bp) deletion of *FeDREB1* promoter activated the expression of the *GUS* genes at low temperatures, but the other 3 deletions did not show this characteristic. To more precisely measure *GUS* expression, we performed quantitative GUS assays. Figure 5A shows that as well as the normal growing condition, under 4°C cold treatment, the activity of GUS in which the tobacco leaves were transformed with *pCD2::GUS*, *pCD3::GUS*, and *pCD4::GUS* vectors showed no detectable activity. However, the activity of *pCD1::GUS* increased significantly compared with the control. The GUS activity of the *pCD1::GUS* transformed tobacco was 32.6% that of the *pC35S::GUS* transformed tobacco (Figure 5A).

Figure 1. Sequence analysis of the *FeDREB1* promoter. Motifs with significant similarity to previously identified *cis*-acting elements are shaded and the names are given under each element. The ATG translation initiation codon is assigned as position 1 in the nucleotide sequence, and the nucleotide positions upstream of position 1 are presented as negative numbers.

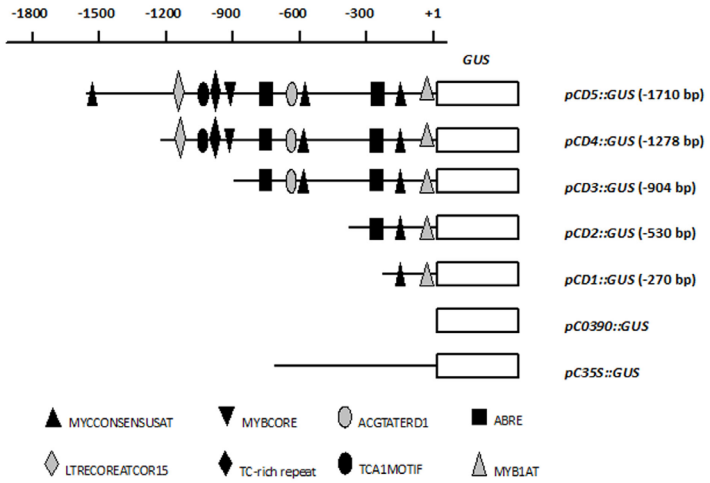

Figure 2. Schematic of *FeDREB1* promoter deletion-GUS constructs. Promoter fragments of different sizes were inserted into the *pC0390GUS* vector containing the GUS reporter gene.

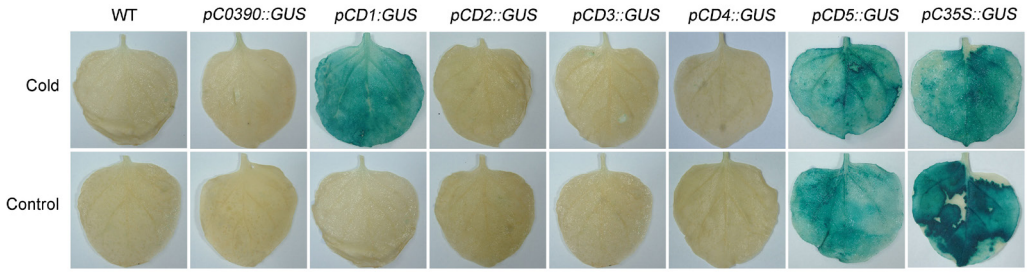

Figure 3. Histochemical assay for GUS expression in the transiently transformed tobacco leaves in plants treated with cold for 24 h. The 7-week tobacco fully expanded leaves were agro-infiltrated with deletion constructs at a concentration of OD_{600} 0.6. GUS staining was performed 24 h after treatment at 4°C (cold) or after treatment at 25°C (control). WT represents wild-type, *pC0390::GUS* represents the negative control, and *pC35S::GUS* represents the positive control.

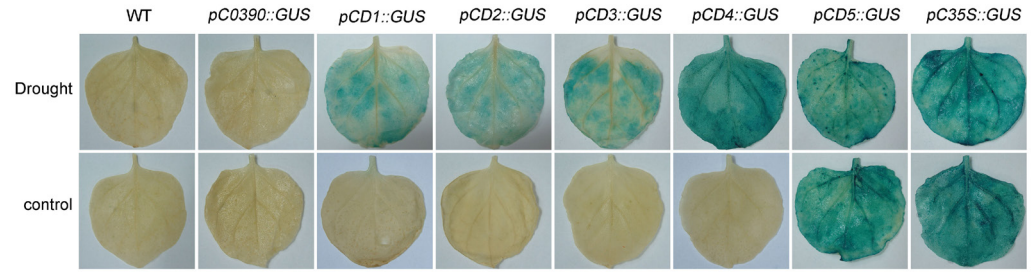

Figure 4. Histochemical assay for GUS expression in the transiently transformed tobacco leaves in plants treated with drought for 24 h. The 7-week tobacco fully expanded leaves were agro-infiltrated with deletion constructs at a concentration of OD_{600} 0.6. GUS staining was performed 24 h after treatment with drought or after treatment at 25°C (control). WT represents wild-type, *pC0390::GUS* represents the negative control, and *pC35S::GUS* represents the positive control.

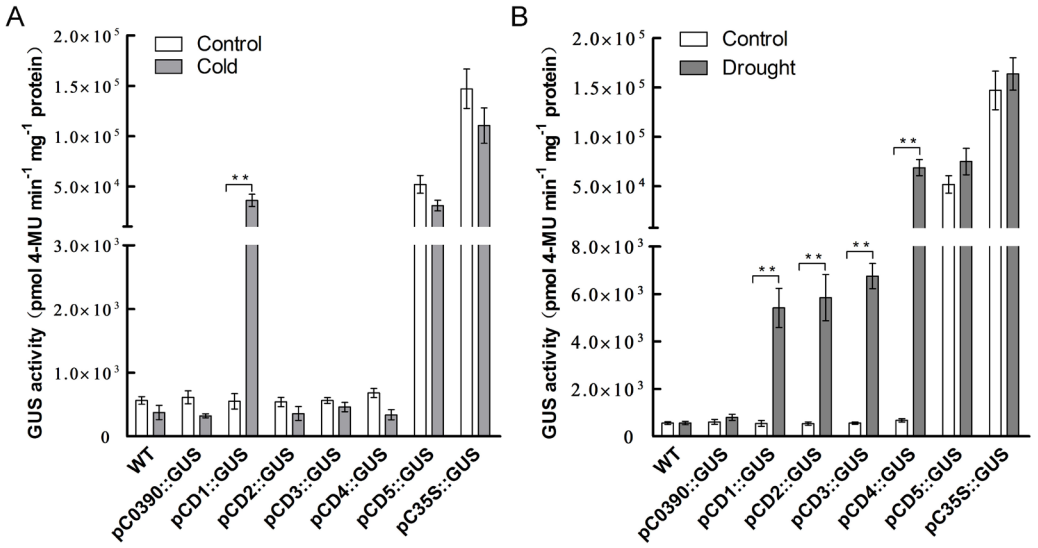

Figure 5. GUS activity driven by *FeDREB1* promoter deletion-GUS chimeric constructs in tobacco leaf tissues. The leaves were infiltrated with each of the deletion plasmids, *pC0390::GUS*, or *pC35S::GUS*, and then treated with cold stress for 24 h (**A**), or treated with drought stress (**B**). The plasmid *pC0390::GUS* was used as a negative control and the *pC35S::GUS* construct was used as a positive control. Data are mean values from 3 independent experiments (N = 3) and error bars represent the standard deviations of the replicates. A one-side paired Student *t*-test was performed to assess significant differences between control and treatment conditions using a statistical software package. Differences at $P < 0.01$(**) were considered significant.

Under drought stress, the tobacco leaves transformed with *pCD1::GUS*, *pCD2::GUS*, and *pCD3::GUS* were blue, while those transformed with *pCD4::GUS* were darker blue (Figure 4). Similar to the results of the histochemical GUS assays, GUS activity of the *pCD1::GUS*, *pCD2::GUS*, *pCD3::GUS*, and *pCD4::GUS* transformed tobacco leaves were remarkably different from the control, and the activity of the induced GUS consistently increased, with *pCD4::GUS* showing the greatest activity (Figure 5B). Under the drought condition, the GUS activity of the *pCD4::GUS* transformed tobacco was 42.0% that of the *pC35S::GUS* transformed activity (Figure 5B). The results of both histochemical and fluorometric assays showed no significant difference in the GUS activity of *pCD5::GUS* transformed tobacco leaves between drought stress and the control, and between cold and the control (Figure 5B).

DISCUSSION

In this study, the *FeDREB1* promoter fragment up to 1710 bp was isolated from the common buckwheat accession Xinong 9976. The result showed that some 5'-deletions of the *FeDREB1* promoter dramatically activated the expression of *GUS* genes under drought or cold stress, indicating that the promoter activity of *FeDREB1* induced expression under drought or cold stress. In addition, among the *FeDREB1* promoter region, the *cis*-acting elements responsive to low temperature and drought were the main components; this may be why the *FeDREB1* promoter acted as a stress-inducible promoter. There are numerous TATA-box and

CAAT-box sequences present in the *FeDREB1* promoter region, which may contribute to the high activity of the *FeDREB1* promoter when efficiently responding to drought or low temperature. Walther et al. (2007) reported that the promoter of genes that can respond to adversity are typically rich in TATA-boxes, which can enhance the capacity of adversity-related genes to respond to abiotic stresses in a more efficient and rapid manner (Roelofs et al., 2010; Yu et al., 2013). In the *pCD1* (-270 bp) promoter region, there are 2 MYCCONSENSUSAT elements. These 2 MYCCONSENSUSAT *cis*-acting elements in the *pCD1* (-270 bp) promoter deletion may be closely related to the expression of *GUS* genes when induced by low temperature in the infiltrated tobacco leaves. In *Arabidopsis*, the MYCCONSENSUSAT motif can bind to MYC-like bHLH protein to activate the expression of the *CBF2/DREB1C* and *CBF3/DREB* genes (Chinnusamy et al., 2003; Zarka et al., 2003). However, whether the expression of *FeDREB1* in common buckwheat is regulated by the MYC-like bHLH protein remains unclear. Under drought treatment, the *pCD1* (-270 bp), *pCD2* (-530 bp), and *pCD3* (-940 bp) deletions showed a gradual increase in the expression of GUS. However, the inductive activity of *pCD4* (-1278 bp) showed a sudden augmentation, suggesting that in the upstream -1278-bp fragment of *FeDREB1*, *cis*-acting elements are responsive to drought stress distributed in sequence, particularly between the -940 and -1278 bp regions; very important drought *cis*-acting elements are also located here. Within the *pCD4* (-1278 bp) deletion, *cis*-acting elements directly related to the drought response, such as MYB1AT, ACGTATERD1, and MYBCORE, were present, which may accumulate and be a vital cause of GUS expression, which gradually improved under the drought condition (Figures 1 and 2). Further analysis indicated that a MYBCORE drought response regulation element was located in the *FeDREB1* promoter between -904 and -1278 bp. Thus, the MYBCORE motif may be a main cause of increased expression of the *GUS* gene under drought conditions. Urao et al. (1993) reported that the MYBCORE motif serves as a recognition site for the ATMYB1 and ATMYB2 proteins in the MYB family. This suggests that MYBCORE is a key responsive *cis*-acting element for drought stress, which may contribute to gene expression under drought conditions. Therefore, the MYB transcription factor may play an important role in *FeDREB1* gene expression under drought stress in common buckwheat. However, a TC-rich repeat sequence is presence within the -904 to -1278 bp region, it remains unclear whether these elements participate in the response process. The *pCD5* (-1710 bp) transformed tobacco leaves both sharply increased the expression of *GUS* genes under the normal control environment as well as under low temperature and drought stresses, indicating that the isolated *FeDREB1* promoter corresponding to drought and low temperature induction should be less than -1710 bp; however, the specific length requires further investigation.

In this study, we successfully separated the promoter of the *FeDREB1* gene, which could respond to low temperature and drought induction. *pCD1* (-270 bp) is the promoter region involved when FeDREB1 engages in the response to low temperature; *pCD4* (-1278 bp) is important when *FeDREB1* is involved during the response to drought stress. Further studies of the inductive promoter of the *FeDREB1* gene would provide novel sources for the breeding of drought-tolerant and cold-resistant plants as well as for plant genetic engineering.

ACKNOWLEDGMENTS

We thank Dr. Xu Weirong (School of Agriculture, Ningxia University) for providing the *pC0390::GUS* and *pC35S::GUS* vectors. Research supported by the National Nature

Science Foundation of China (Grant #31071472) and the Special Fund for Agro-scientific Research in the Public Interest (Grant #201303008).

REFERENCES

Agarwal M, Hao Y, Kapoor A, Dong C-H, et al. (2006a). A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. *J. Biol. Chem.* 281: 37636-37645.

Agarwal PK, Agarwal P, Reddy M and Sopory SK (2006b). Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant Cell Rep.* 25: 1263-1274.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.

Chen H, Je J, Song C, Hwang JE, et al. (2012). A proximal promoter region of *Arabidopsis DREB2C* confers tissue-specific expression under heat stress. *J. Integr. Plant Biol.* 54: 640-651.

Chen M, Xu Z, Xia L, Li L, et al. (2009). Cold-induced modulation and functional analyses of the DRE-binding transcription factor gene, *GmDREB3*, in soybean (*Glycine max* L.). *J. Exp. Bot.* 60: 121-135.

Chinnusamy V, Ohta M, Kanrar S, Lee B-h, et al. (2003). *ICE1*: a regulator of cold-induced transcriptome and freezing tolerance in *Arabidopsis*. *Genes Dev.* 17: 1043-1054.

Cook D, Fowler S, Fiehn O and Thomashow MF (2004). A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 101: 15243-15248.

Dietz K-J, Vogel MO and Viehhauser A (2010). AP2/EREBP transcription factors are part of gene regulatory networks and integrate metabolic, hormonal and environmental signals in stress acclimation and retrograde signalling. *Protoplasma* 245: 3-14.

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, et al. (2003). *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high salt -, and cold-responsive gene expression. *Plant J.* 33: 751-763.

Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, et al. (1998). Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced *COR* gene expression. *Plant J.* 16: 433-442.

Gong Z, Lee H, Xiong L, Jagendorf A, et al. (2002). RNA helicase-like protein as an early regulator of transcription factors for plant chilling and freezing tolerance. *Proc. Natl. Acad. Sci. U. S. A.* 99: 11507-11512.

Higo K, Ugawa Y, Iwamoto M and Korenaga T (1999). Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Res.* 27: 297-300.

Ito Y, Katsura K, Maruyama K, Taji T, et al. (2006). Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol.* 47: 141-153.

Jefferson RA (1987). Assaying chimeric genes in plants: the *GUS* gene fusion system. *Plant Mol. Biol. Rep.* 5: 387-405.

Kizis D (2002). Maize DRE-binding proteins DBF1 and DBF2 are involved in rab17 regulation through the drought-responsive element in an ABA-dependent pathway. *Plant J.* 30: 679-689.

Lee H, Xiong L, Gong Z, Ishitani M, et al. (2001). The *Arabidopsis HOS1* gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleo-cytoplasmic partitioning. *Genes Dev.* 15: 912-924.

Lescot M, Déhais P, Thijs G, Marchal K, et al. (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* 30: 325-327.

Liu Q, Kasuga M, Sakuma Y, Abe H, et al. (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought-and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* 10: 1391-1406.

Qiao Z-X, Huang B and Liu J-Y (2008). Molecular cloning and functional analysis of an ERF gene from cotton (*Gossypium hirsutum*). *Biochim. Biophys. Acta* 1779: 122-127.

Qin F, Sakuma Y, Li J, Liu Q, et al. (2004). Cloning and functional analysis of a novel DREB1/CBF transcription factor involved in cold-responsive gene expression in *Zea mays* L. *Plant Cell Physiol.* 45: 1042-1052.

Rabbani MA, Maruyama K, Abe H, Khan MA, et al. (2003). Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiol.* 133: 1755-1767.

Ramanjulu S and Bartels D (2002). Drought-and desiccation-induced modulation of gene expression in plants. *Plant Cell Environ.* 25: 141-151.

Roelofs D, Morgan J and Stürzenbaum S (2010). The significance of genome-wide transcriptional regulation in the evolution of stress tolerance. *Evol. Ecol.* 24: 527-539.

Rushton PJ, Torres JT, Parniske M, Wernert P, et al. (1996). Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley *PR1* genes. *EMBO J.* 15: 5690.

Shen YG, Zhang WK, He SJ, Zhang JS, et al. (2003). An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration and ABA stress. *Theor. Appl. Genet.* 106: 923-930.

Shinozaki K and Yamaguchi-Shinozaki K (2000). Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Curr. Opin. Plant Biol.* 3: 217-223.

Shinwari ZK, Nakashima K, Miura S, Kasuga M, et al. (1998). An *Arabidopsis* Gene Family Encoding DRE/CRT Binding Proteins Involved in Low-Temperature-Responsive Gene Expression. *Biochem. Biophys. Res. Commun.* 250: 161-170.

Sparkes IA, Runions J, Kearns A and Hawes C (2006). Rapid, transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants. *Nat. Protoc.* 1: 2019-2025.

Tian XH, Li XP, Zhou HL, Zhang JS, et al. (2005). *OsDREB4* Genes in Rice Encode AP2-Containing Proteins that Bind Specifically to the Dehydration-Responsive Element. *J. Interg. Plant Biol.* 47: 467-476.

Urao T, Yamaguchi-Shinozaki K, Urao S and Shinozaki K (1993). An *Arabidopsis* myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. *Plant Cell*: 1529-1539.

Walther D, Brunnemann R and Selbig J (2007). The regulatory code for transcriptional response diversity and its relation to genome structural properties in *A. thaliana*. *PLoS Genet.* 3: e11.

Xiong L, Lee H, Ishitani M, Tanaka Y, et al. (2002). Repression of stress-responsive genes by FIERY2, a novel transcriptional regulator in *Arabidopsis*. *Proc Natl. Acad. Sci. U. S. A.* 99: 10899-10904.

Xu W, Yu Y, Ding J, Hua Z, et al. (2010). Characterization of a novel stilbene synthase promoter involved in pathogen-and stress-inducible expression from Chinese wild *Vitis pseudoreticulata*. *Planta* 231: 475-487.

Yamaguchi-Shinozaki K and Shinozaki K (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* 57: 781-803.

Yu Y, Xu W, Wang J, Wang L, et al. (2013) A core functional region of the *RFP1* promoter from Chinese wild grapevine is activated by powdery mildew pathogen and heat stress. *Planta* 237: 293-303.

Zarka DG, Vogel JT, Cook D and Thomashow MF (2003). Cold induction of *Arabidopsis* CBF genes involves multiple *ICE* (inducer of CBF expression) promoter elements and a cold-regulatory circuit that is desensitized by low temperature. *Plant Physiol.* 133: 910-918.