Theor Appl Genet (2003) 106:923–930
DOI 10.1007/s00122-002-1131-x

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An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration and ABA stress

Received: 21 June 2002 / Accepted: 5 August 2002 / Published online: 15 November 2002

Abstract We characterize one transcription factor of DRE-binding proteins (TaDREB1) that was isolated from a drought-induced cDNA library of wheat (*Triticum aestivum* L.). TaDREB1 contains one conserved EREBP/AP2 domain, and shows similarity with *Arabidopsis thaliana* DREB family members in both overall amino-acid sequences and the secondary structure arrangement within the DNA-binding motifs. In yeast one-hybrid system, TaDREB1, can specially activate the genes fused with the promoter containing three tandemly repeated copies of the wild-type DRE sequence: TACCGACAT. In different wheat cultivars, the *Ta DREB1* gene is induced by low temperature, salinity and drought; and the expression of *Wcs120* that contains DRE motifs in its promoter is closely related to the expression of *TaDREB1*. These results suggest that TaDREB1 functions as a DRE-binding transcription factor in wheat. We also observed the dwarf phenotype in transgenic rice (T0) overexpressing *TaDREB1*.

Keywords DRE-binding protein · One-hybrid · Abiotic stresses · *Triticum aestivum* L.

Introduction

Adverse environmental stresses such as drought, salinity and cold cause a great loss in the crop yield every year all over the globe (Boyer 1982). To improve crop tolerance to these abiotic stresses, researches have been focused on the physiological and molecular mechanisms of plant responses to the stresses. Molecules that function in adaptation to environmental stresses can be divided into two groups: the effector molecules and the regulatory molecules (Hasegawa et al. 2000). The hydrophilic protein, for example *rd29A*, *cor15A* and *erd10* from *Arabidopsis*, *wcs120* from wheat and *blt14* from barley (Skriver and Mundy 1990; Houde et al. 1992; Dunn et al. 1994; Ingram and Bartels 1996), is one of the most important effector molecules that had been identified from various plant species (Thomashow 1999). These proteins are composed of repeated amino-acid sequence motifs and remain soluble upon boiling. In both monocotyledon and dicotyledon plants, hydrophilic proteins can increase freezing tolerance through protecting choloroplasts against membrane leakage caused by freezing (Steponkus et al. 1998).

In Arabidopsis, expression of *cor* and *rd* genes is quickly induced by low temperature or dehydration and subsequently reduced after being released from stress conditions. Exogenous abscisic (ABA) also has a slow effect on the induction of *cor* genes, indicating that both ABA-dependent and dehydration-dependent pathways are involved in the regulation of *cor* expression. Functional analysis of the promoter regions of these stress-inducible and ABA-inducible genes, like *rd29A* and *cor15A*, leads to the identification of two *cis*-elements, the Abscisic acid-responsive-element (ABRE) and the Dehydration-responsive-element (DRE), which are responsible for ABA-inducible and dehydration-inducible transcription, respectively (Yamaguchi-Shinozaki and Shinozaki 1994; Jiang et al. 1996; Ouellet et al. 1998). Regulation of effector molecules through DRE was then found to be involved in stress signal transduction pathways in Arabidopsis (Ishitani et al. 1997; Knight et al. 1999; Lee et al. 2001). Recently, genes encoding transcription factors that specifically bind DRE have been cloned fromArabidopsis by using the yeast one-hybrid screening technique (Stockinger et al. 1997; Gilmour et al. 1998; Liu et al. 1998; Medina et al. 1999). They belong to a large DNA-binding protein family containing a

Communicated by H.F. Linskens

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conserved EREBP/AP2 domain. However, different members of this family regulate different processes such as plant development, stress response and hormone responses etc. (Weigel 1995; Riechmann and Meyerowitz 1998). Expression of the *CBF/DREB1* gene is strongly induced by low-temperature, whereas expression of the *DREB2* gene is induced by dehydration. The results indicate that two independent families of DRE-binding proteins function as *trans*-acting factors in two separate signal transduction pathways under low-temperature and dehydration conditions, respectively (Liu et al. 1998).

In Asia, growth and productivity of crops, especially those primary crops such as rice and wheat, suffered from dehydration and freezing. The molecular mechanism of stress tolerance in monocotyledons is poorly understood. Here we characterized one regulatory molecule, the DRE-binding transcription factor (TaDREB1), that mainly responded to freezing. The expression patterns of *TaDREB1* and a putative downstream gene *wcs120* were investigated in two cultivars with different freezing tolerance.

Materials and methods

Plant materials, growth conditions and stress treatments

Seeds of winter wheat (*Triticum aestivum* L. cv Xiaoyan54) and spring wheat (*T. aestivum* L. cv Chinese Spring) were germinated at 37 °C overnight and grown hydroponically at 25 °C with a photoperiod of 12 h. After 10 days, some seedlings were removed and grown at 0 °C with a photoperiod of 12 h for cold treatment; and some seedlings were transferred into solutions containing 200 mM of NaCl, 25 μM of ABA or 20% PEG6000 for salinity, ABA, or drought-stress treatment, respectively. The two cultivar seedlings of control and stress-treated for different times were harvested, quickly immersed in liquid nitrogen and stored at –70 °C for DNA and RNA extraction.

Construction and screening of a cDNA library

Using a poly (A^+) mRNA purification kit (Promega), poly(A^+) RNA was isolated from leaves of 10-day old seedlings of wheat (*T. aestivum* L. cv Xiaoyan54) that were dehydrated on Whatman 3MM paper at 25 °C for 8 h. Two micrograms of poly (A^+) RNA were used for cDNA library construction as described previously (Zhang et al. 2001). Approximately 500,000 plaques were screened with *Arabidopsis* DREB2A cDNA as probes. One positive plaque was obtained from the third-round screening and the insert in the recombinant phage was excised in vivo into the pExCell plasmid (Amersham) following the instructions. The insert was subjected to sequencing analysis.

In vivo DRE-binding and transactivation experiment by using the yeast one-hybrid system

To analyze DRE-binding activity and transactivation activity of the isolated cDNA clone, the full-length cDNA insert was cloned into the yeast expression vector YepGAP (Fig. 4A) under the promoter of the glyceraldehyde 3-phosphate dehydrogenase gene and the terminator of the alcohol dehydrogenase 1 gene. This plasmid was transformed into two yeast strains following the manufacturer's instruction (Clontech), one carrying the dual reporter genes *HIS3* and *lacZ* that had been fused to 71-bp DNA fragments of the *rd29A* promoter containing the DRE sequence, and the other carrying the same construct but with four base substitutions in the DRE sequence (Fig. 4A). After both yeast transformants grew up on selective medium without Ura and Tyr, these two transformants were analyzed on selective medium plates without His, Ura and Tyr but containing 10-, 20-, 30- and 60-mM 3-AT (a competitive inhibitor of the *HIS3* gene product) to test the expression of the *HIS3* gene. The β-galactosidase activities of the survival clones were analyzed as referred to the manufacturer's instruction (Clontech). The yeast expression vector YepGAP, and the DRE and mDRE yeast strains, were described as previously by Liu et al. (1998).

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   1 CAAAACCAAGGCGGCGGCAGCGGGGCGGGAGAGCGGGGAGCACCGACCGACACCGGCCGACAGGGTGGGCTGCAT
  76 GCGGAGCTGAGGCGAGGCGAGGCGAGGCGGGGAGAGATCCGGCGCGGGTGCCACCGCCGGCCGGCCGCGGGAGAT
 151 CTGGTTGGTGGCGCCGCCCGGATAAGGGAGAGGCGGCGAGGGGAGAGCAGCCGGGGGAGACCGAGGCGAGAGGAG
 226 ATCTCTCTCGTCCCTCTTCTCGCTCCATGGAGACCGGGGGTAGCAAGCGGGAAGGAGACTGCCCCGGGCAGGAAA
                                M  E  T  G  G  S  K  R  E  G  D  C  P  G  Q  E  R
 301 GGAAGAAGAAAGTGCGCAGGAGAAGCACTGGTCCTGATTCGGTTGCTGAAACCATCAAGAAGTGGAAGGAGGAAA
       K  K  K  V  R  R  R  S  T  G  P  D  S  V  A  E  T  I  K  K  W  K  E  E  N
 376 ACCAGAAGCTCCAGCAAGAGAATGGATCCCGGAAAGCACCGGCCAAGGGTTCCAAGAAAGGGTGCATGGCAGGGA
       Q  K  L  Q  Q  E  N  G  S  R  K  A  P  A  K  G  S  K  K  G  C  M  A  G  K
 451 AAGGAGGTCCAGAGAATTCAAACTGCGCTTACCGCGGTGTGAGGCAGAGGACGTGGGGGAAATGGGTTGCTGAGA
       G  G  P  E  N  S  N  C  A  Y  R  G  V  R  Q  R  T  W  G  K  W  V  A  E  I
 526 TCCGTGAGCCCAACCGTGGCAATCGGCTGTGGCTTGGTTCATTCCCTACCGCAGTCGAAGCTGCACGTGCATATG
       R  E  P  N  R  G  N  R  L  W  L  G  S  F  P  T  A  V  E  A  A  R  A  Y  D
 601 ATGATGCGGCAAGGGCAATGTATGGCGCCAAAGCACGTGTCAACTTCTCAGAGCAGTCCCCGGATGCCAACTCTG
       D  A  A  R  A  M  Y  G  A  K  A  R  V  N  F  S  E  Q  S  P  D  A  N  S  G
 676 GTTGCACGCTGGCACCTCCATTGCCGATGTCTAATGGGGCAACCGCTGCGTCACATCCTTCTGATGGGAAGGATG
       C  T  L  A  P  P  L  P  M  S  N  G  A  T  A  A  S  H  P  S  D  G  K  D  E
 751 AATCGGAGTCTCCTCCTTCTCTTATCTCAAATGCGCCGACAGCTGCGCTGCATCGGTCTGATGCTAAGGATGAGT
       S  E  S  P  P  S  L  I  S  N  A  P  T  A  A  L  H  R  S  D  A  K  D  E  S
 826 CTGAGTCTGCAGGGACCGTGGCACGTAAGGTGAAAAAAGAAGTGAGCAATGATTTGAGAAGTACCCATGAGGAGC
       E  S  A  G  T  V  A  R  K  V  K  K  E  V  S  N  D  L  R  S  T  H  E  E  H
 901 ACAAGACCCTGGAAGTATCCCAACCAAAAGGGAAGGCTTTACATAAAGCAGCGAACGTAAGTTATGATTACTTCA
       K  T  L  E  V  S  Q  P  K  G  K  A  L  H  K  A  A  N  V  S  Y  D  Y  F  N
 976 ACGTCGAGGAAGTTCTTGACATGATAATTGTGGAATTGAGTGCTGATGTAAAAATGGAAGCACATGAAGAGTACC
       V  E  E  V  L  D  M  I  I  V  E  L  S  A  D  V  K  M  E  A  H  E  E  Y  Q
1051 AAGATGGTGATGATGGGTTTAGTCTTTTCTCATATTAGGGTTTTAGCTATGAGGGTTGCAGTCATGCGGAGCAAT
       D  G  D  D  G  F  S  L  F  S  Y  *
1126 AGGGATAACTTTCATTCTAGCTGCTAGGAAATACTTCAAATCTGCAACCCGAAGCTTTGTAGTCACTTATGGTTT
1201 TCATCTTACTGGAGAGAATAGCTTTATACCATAAGTCAACGGGTACAAGAAGTTGTCCTGTGCGTTGAGTTCATG
1276 TACTATGGTAAAAGTTG
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Fig. 1 Nucleotide sequence and deduced amino-acid sequence of DREB1 of wheat (*Triticum aestivum* L.). The EREBP/AP2 domain is *underlined*, the basic region in the N-terminal region that might function as a nuclear localization signal is *underlined* by a dash line, and a putative Ser/Thr-rich region followed the EREBP/AP2 domain is indicated by the second *dashed underline* This sequence has been deposited in GenBank databases under the accession number AF303376

DNA sequencing and data analysis

DNA sequences were determined using the *Taq* Dye Primer Cycle Sequencing Kit (Amersham) and the ABI 373A automatic sequencer. The nucleotide and amino-acid sequences were compared with those released in GenBank databases by using the GAPPED BLAST analysis program. The full-length sequence of *TaDREB1* has been deposited in GenBank databases under the accession number AF303376. The alignment report was produced by the DNASTAR software. The DOS program designed on the principles and models described (Jones et al. 1992; Okamuro et al. 1997) was used for prediction of the amphipathic α-helix domain.

RNA gel blot and reverse transcription-PCR (RT-PCR) analysis

Total RNA extraction, Northern blotting and filter washing conditions were as described by Li and Chen (2000). When using RNA

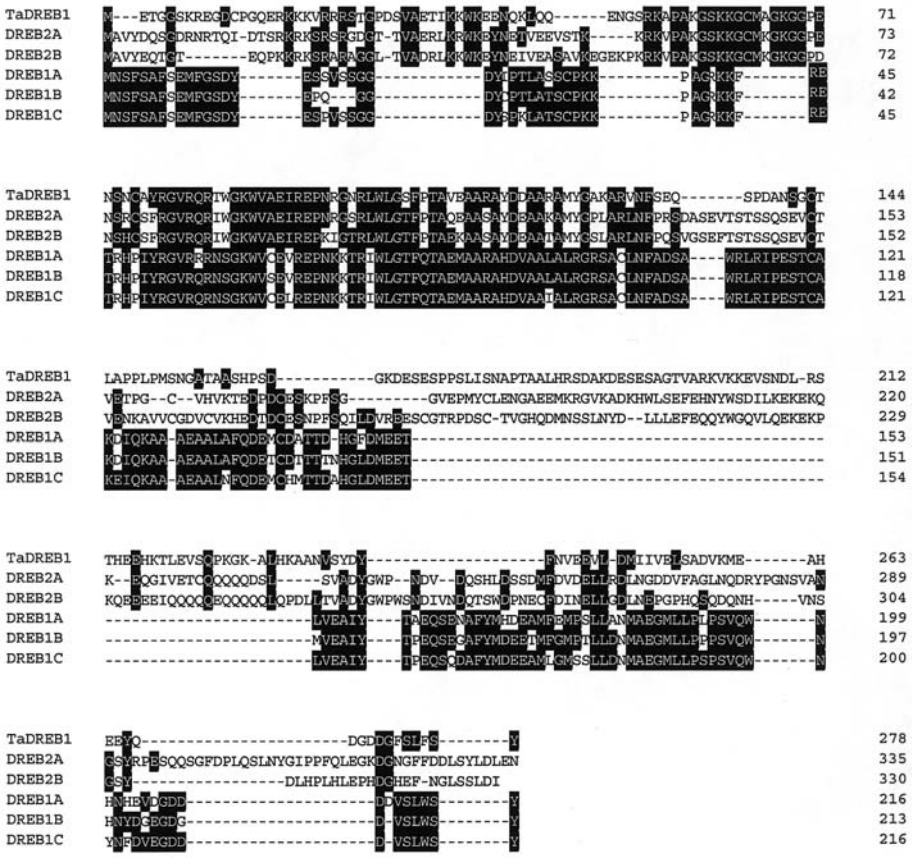

Fig. 2 Comparison of the deduced amino-acid sequence of TaDREB1 with AtDREB1A and AtDREB2A. Gaps are introduced to maximize the similarities, *Shaded residues* indicate the identity. The conserved EREBP/AP2 domains are *underlined*. The *asterisks* mark the two residues, "V" and "E" which had been especially identified (Liu et al. 1998; Cao et al. 2001)

gel blotting, wcs120 cDNA and the 18s rDNA gene were labeled with α-^{32}P-dCTP (Amersham) as probes. When employing RT-PCR, 5 µg of total RNA of each sample was digested with DNase I (Promega) and used for first-strand cDNA synthesis (Gibco). A pair of gene-specific primers 5′-ATGGAGACCGGGGGTAGC-3′ and 5′-CTAATATGAGAAAAGACTAAAC-3′, and a pair of primers for a part of the constitutively expressed actin gene *RAc1* (McElroy et al. 1990), were designed for PCR amplification using first-strand cDNA as a template. The PCR condition was 94 °C for 3 min and 35 cycles of 94 °C for 30 s, 54 °C for 1 min, and 72 °C for 1 min, with a final extension of 10 min at 72 °C. PCR Products were separated on a 1% agarose gel containing ethidium bromide and photographed. The signals were quantified by using the Imaging DensitoMeter (Model GS-670 Bio-Rad).

Generation of transgenic plants

A *Sma*I/*Sac*I fragment of the TaDREB1 cDNA was ligated into the *Sma*I/*Sac*I site of the binary vector pCambia1300 (Clontech) under the control of the 35S promoter. Mature seed–derived rice callus (*Oryza sativa* L. ssp. *indica* cv Zhonghua 9) was used for particle bombardment and regenerated under 50 mg/l of hygromycin selection. After differentiation, two independent lines were obtained and confirmed by Southern and Northern blots. Plants of control and transgenic lines were moved to a soil tank and cultured in the green house at 22 °C under natural daylight during August and September, and the growth state was photographed every week. Transgenic Arabidopsis was obtained by the vacuum method and 20 independent T2 lines were used for RNA analysis.

Results

Isolation and characterization of the TaDREB1 gene

To isolate the genes encoding DRE-binding proteins from wheat, seedlings were desiccated on filter paper to construct a dehydration-induced cDNA library. One clone was isolated from 500,000 plaques by using AtDREB2A cDNA as a probe. Sequence analysis showed that the cDNA insertion was 1.3 kb in length, including a complete open reading frame of 837 bp with a 5′-UTR of 251 bp and a 3′-UTR of 204 bp, and named *TaDREB1* (Fig. 1). *TaDREB1* encoded a polypeptide of 278 amino acids with a calculated molecular weight of 30.3 kDa. Analysis of the deduced amino-acid sequence revealed that this protein contained one conserved DNA binding domain of 68 amino acids that was named as the EREBP/AP2 domain in a large family of plant DNA binding proteins. A conserved nuclear localization signal was found in the N terminal of TaDREB1, and a conserved Ser/Thr-rich region was adjacent to the EREBP/AP2 DNA binding domain. A homology search of GenBank databases showed that TaDREB1 only has 33% similarity with AtDREB2A in amino-acid sequence (Fig. 2). However, the EREBP/AP2 domain of TaDREB1 exhibited 50.0% and 67.6% similarity to those of the AtDREB1A/CBF1 and AtDREB2A (Fig. 3A), respectively.

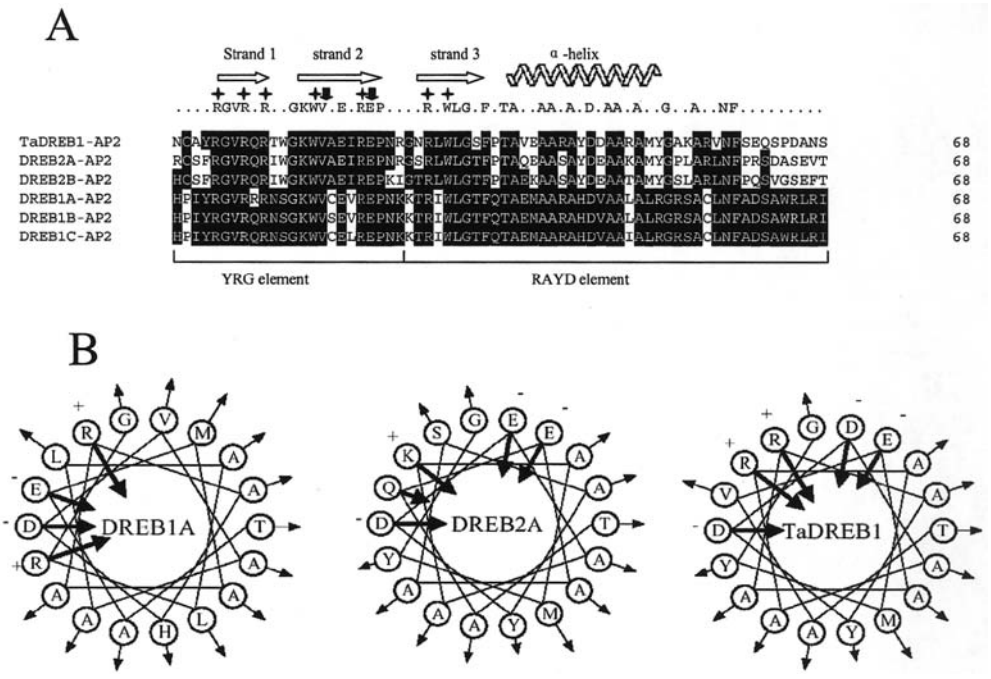

Fig. 3A, B Comparision of EREBP/AP2 domain sequences and structures of TaDREB1, AtDREB1A and AtDREB2A **A** Sequence alignment of the EREBP/AP2 domains. Each EREBP/AP2 domain had 68 amino acids, sequence alignment was as described previously, conserved residues are concluded above, *asterisks above* indicated the residues with important functions as discussed, three β-sheets and the amphipathic α-hilex are marked above the corresponding sequences as a reference (Allen et al. 1998). *Brackets* below showed the location of the conserved YRG and RAYD elements that were determined as to the reference (Okamuro et al. 1997). **B** Schematic diagrams of the putative amphipathic a-helices of TaDREB1, AtDREB1A and AtDREB2A. The 18 amino-acid residues within the RAYD elements were predicted to form amphipathic a-helices. In the current schematics, each residue rotates clockwise by 100° to form the helical structure; a negative or positive degree of hydrophobicity was shown by *arrows* directed toward or away from the center of the helical wheel diagrams; positively and negatively charged amino-acid residues are represented by + and – signs, respectively

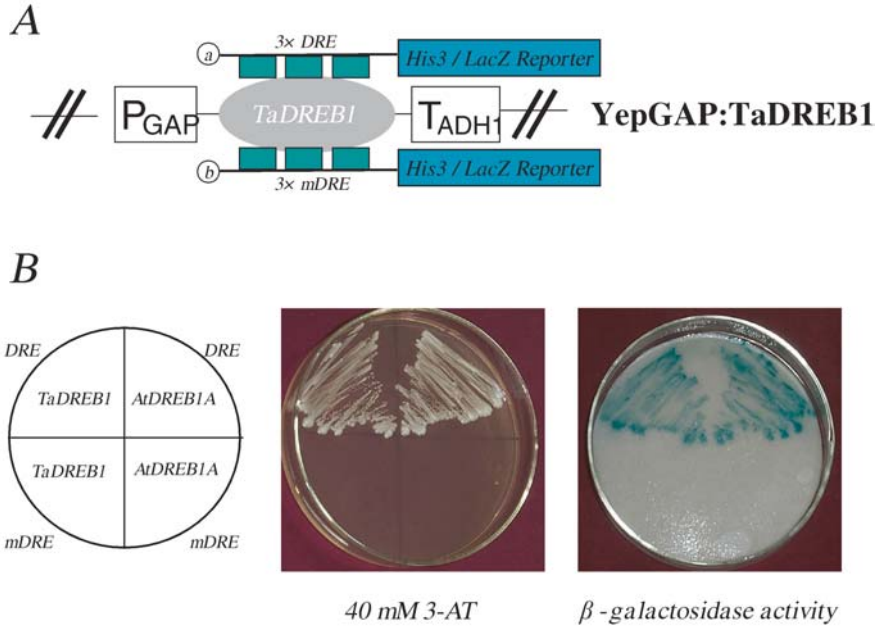

Fig. 4A, B TaDREB1 transactivated the dual reporter genes in yeast one hybrid system. **A** The TaDREB1 cDNA was cloned into the yeast expression vector YepGAP and used for transformation into DRE yeast and mDRE yeast as described in Materials and methods. **B** Heterogenous expression of the *TaDREB1* gene in yeast strains carrying the dual reporter genes under the control of the DRE motifs or mDRE motifs. The transformants were examined for growth in the presence of 3-AT and β-galactosidase activity.

Sequences within the EREBP/AP2 domain are recently reported to form a three-stranded anti-parallel β-sheet and a α-helix when combining with the target DNA fragment. The residues, "A" in strand 2, "LG" in the strand 3 and "RW" in all β-sheets were almost identical among the three proteins, and may be important for the structure of the binding complex (Allen et al. 1998). The 18 amino-acid core region within the RAYD element of the EREBP/AP2 domain is predicted to form an amphipathic α-helix that may be important for interaction with an-

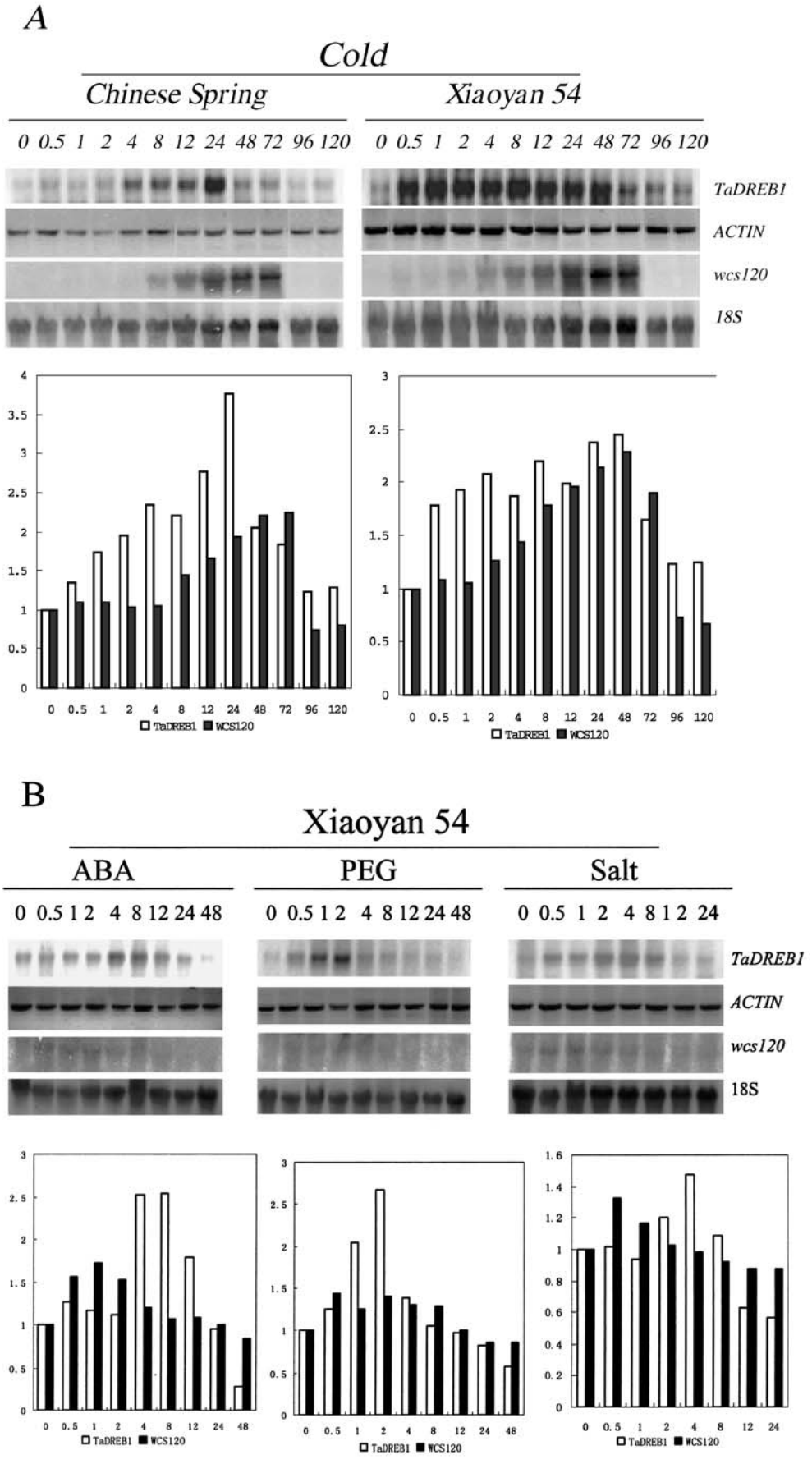

Fig. 5 Time-course expression analysis of *TaDREB1* in wheat seedlings in response to cold (**A**), 25 μM ABA, 20% PEG6000 and 200 mM NaCl (**B**). The *TaDREB1* transcripts between Xiaoyan 54 and Chinese Spring wheat varieties under cold stress were compared. RT-PCR assay and Northern blots were performed as described in Materials and methods, *Wcs120* (M93342) was probed as a DRE-related marker gene. The signals were quantitated and the ratio of *TaDREB1* band intensities to *ACTIN* band intensities, or *Wcs120* band intensities to 18S rDNA band intensities, represented the relative expression level of the corresponding genes. The quantitation analysis of the *TaDREB1* and *Wcs120* gene expression in response to each stress was placed under the corresponding stresses

other protein or for DNA-binding (Okamuro et al. 1997). We used computer programs to determine whether this secondary structure is conserved in the DRE-binding proteins. Figure 3 B shows that the second structure of TaDREB1 is more similar to that of AtDREB2A than that of AtDREB1A.

Heterogenous expression, DRE-binding and transactivation activity of TaDREB1

To analyze the in vivo function of this putative DRE-binding protein from wheat, the full-length cDNA of TaDREB1 was subcloned into the yeast expression vector YepGAP and transformed into two yeast strains carrying either a wild-type or a mutant DRE, respectively (Fig. 4A). In wild-type DRE yeast, transformants carrying the TaDREB1 plasmid can grow well on the medium lacking histidine in the presence of 10-, 20-, 30- and 60-mM of 3-AT (a competitive inhibitor of the *HIS3* gene product), whereas the transformants of mutant DRE yeast can not grow on the same medium. Together with the results of the β-galactose activity assay shown in (Fig. 4B), it can be seen that heterogenous expression of TaDREB1 promoted expression of the *HIS3* and *lacZ* genes in the wild-type DRE yeast but not in the mutant DRE yeast, indicating that the *TaDREB1* gene encodes a transcription factor that can specifically bind to DRE motifs in promoter regions and activate transcription of the downstream genes.

Expression of *TaDREB1* in response to different abiotic stresses

The RT-PCR method was used to investigate *TaDREB1* transcription in response to low-temperature, ABA, PEG6000 and salt stresses, because expression of *TaDREB1* is too low to be detected by Northern blotting (data not shown). A putative downstream gene *wcs120*, which is cold-inducible and has a GCC box in its promoter region (Vazquez-Tello et al. 1998), was also investigated for its expression under the same conditions. Figure 5 A showed that low-temperature induced much earlier and stronger expression of *TaDREB1* in Xiaoyan 54, a winter wheat, when compared with its expression pattern in Chinese Spring, a spring wheat. Moreover, the induction period was longer in Xiaoyan 54 than in Chinese Spring. Expression of marker gene *wcs120* closely followed the expression patterns of *TaDREB1* in the two cultivars, and reached its highest level at the end of freezing treatment. When the seedlings were removed from the stress and transferred into a non-stressed condition, *wcs120* transcripts quickly disappeared. We also studied the expression of *TaDREB1* and the *Wcs120* gene in Xiaoyan 54 under salt, PEG6000, cold and ABA treatments (Fig. 5B). All treatments induced expression of *TaDREB1*. However, transcription of *wcs120* was only slightly induced by ABA and salt treatment. In addition, expressions of both genes under salt, PEG6000 and ABA

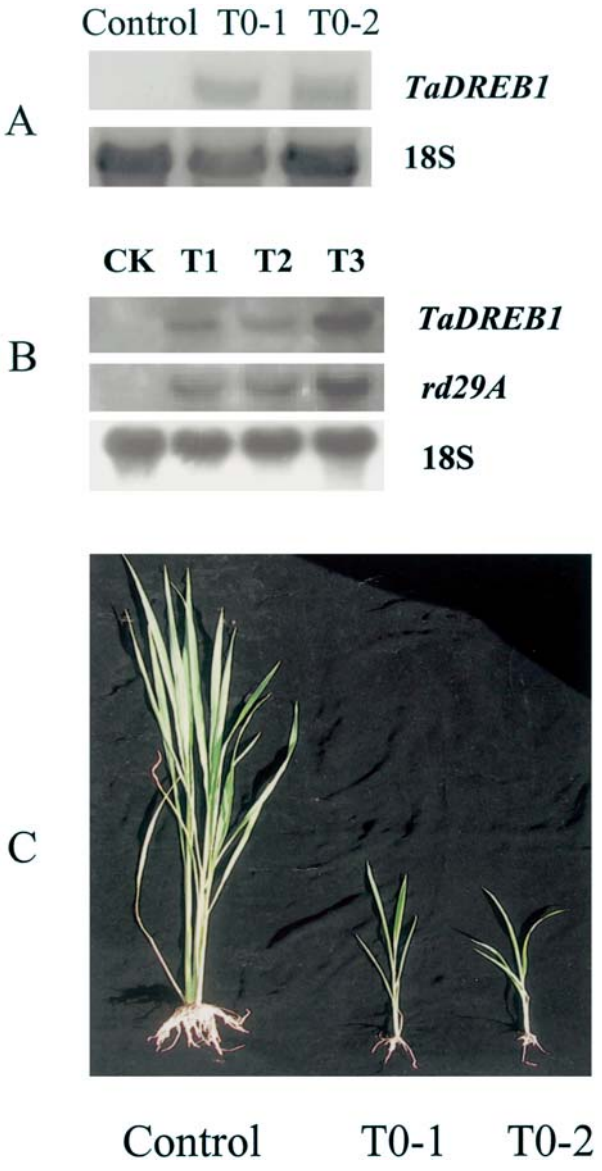

Fig. 6A–C Overexpression of the *TaDREB1* gene in rice caused a dwarf phenotype. **A** Overexpression of the *TaDREB1* gene was analyzed by Northern blotting in transgenic rice. **B** Analysis of *TaDREB1* and *rd29A* expression in transgenic Arabidopsis. **C** The transgenic plant has an obvious lower height than the control plant after transplanting in the soil tank for 2 months. T0-1 and T0-2 indicate the two independent primary transgenic lines 1 and 2

treatments were much less when compared with their expressions under freezing stress.

Phenotype of transgenic plants

To characterize the function of the *TaDREB1* gene in transgenic plants, the *TaDREB1* gene in a sense orientation was inserted into the binary vector pCambia1300 under the control of the 35S promoter, transferred into rice by particle bombardment and into Arabidopsis by vacuum infiltration. Two primary rice transformants and 20 Arabidopsis

T2 lines were obtained after selection on hygromycin media and analyzed by Northern blotting (Fig. 6A, B). Expressions of the putative downstream genes were also determined in transgenic rice and Arabidopsis by using *wcs120* and *rd29A* cDNA respectively, as probes. In Arabidopsis T2 lines, overexpression of the *TaDREB1* gene activated expression of the downstream gene *rd29A* under the non-stress condition (Fig. 6B). However no *wcs120* expression was detected in transgenic rice, probably due to either the low homology between the *wcs120* gene from rice and from wheat, or the actual low expression level of the *wcs120* gene in rice. We also analyzed the phenotype of *TaDREB1* transgenic plants and found that overexpression of the *TaDREB1* gene in Arabidopsis had no negative effect on plant development, as AtDREB1 did (data not shown). However, when overexpressed in rice, plant growth was greatly reduced (Fig. 6C).

Discussion

Isolation and overexpression of AtDREB1/CBF and AtDREB2 in Arabidopsis has brought an exciting foreground for plant genetic engineering of stress tolerance. Overexpression of AtDREB genes under 35S or the stress-inducible promoter could greatly improve stress tolerance in transgenic Arabidopsis. However, untill now few DRE-binding proteins were isolated from other plant species. In the present study we characterized a novel DRE-binding protein isolated from a monocotyledon plant, wheat. After expression in the yeast one-hybrid system, we demonstrated the DRE-binding activity and *trans*-activation activity of the TaDREB1 in vivo. Amino-acid sequence analysis of TaDREB1 indicated a conserved nuclear location signal in the N terminal. In addition, a conserved Ser/Thr-rich region was adjacent to the EREBP/AP2 DNA binding domain, and this region may contain the phosphorylation site for the regulation of TaDREB1 activity (Liu et al. 1998). Previous study on cold response in wheat has found that phosphorylation was essential for the binding of nuclear factors to LTRE/DRE motifs in the *wcs120* promoter (Vazquez-Tello et al. 1998). In DREB2A, a similar Ser/Thr-rich region was identified as a putative interaction domain to be modified by other regulatory molecules in a dehydration signal transduction pathway.

Some residues in the β-sheet have been identified as key residues for the DNA-binding activity of EREBP/AP2-type proteins; for example arginine (R) and tryptophan (W) contacted DNA elements, alanine (A) and glycine (G) made a hydrogen bond (Allen et al. 1998), and mutation of the G in strand 3 caused malfunction of APETALA2 (Jofuku et al. 1994). Besides these residues, there are some other residues in the EREBP/AP2 domain identical among the three DRE-binding proteins (Fig. 3A), such as V in strand 2. Recent experiments found that mutation of V in strand 2 caused on obvious decrease in DRE-binding activity of DREB1A, whereas mutation of E at the end of strand 2 could not (Cao et al. 2001). We suppose that the nucleotides in the DRE sequence distinguished DREB from others through some particular conserved residues, e.g. V in strand 2 which showed a high identity only among the DREB protein category (Liu et al. 1998). It is then necessary to isolate more TaDREB members to identify these particular residues.

In wheat, cold induced the expression of *TaDREB1* and *wcs120*, and showed a different expression pattern in winter wheat and spring wheat. In winter wheat, *TaDREB1* and the *wcs120* gene were induced rapidly and at a relatively higher level. Whereas, in spring wheat, both genes were induced at a relatively low level. The higher level of the two gene expressions may contribute to the cold tolerance in winter wheat. Because the abundance of Wcs120 was closely related to the *TaDREB1* transcripts, it is possible that *TaDREB1* activated the transcription of the *wcs120* gene. When the cold stress was released, the *Wcs120* mRNA was quickly degraded, indicating its instability under normal temperature and its important roles in cold tolerance. In addition to the cold stress, salt and exogenous ABA treatments also induced *TaDREB1* expression, but at a relatively low level. The *wcs120* gene expression, however, was not significantly induced. This phenomenon may indicate that the *wcs120* gene was not the downstream gene for *TaDREB1* under drought, salt and ABA treatments. It is possible that other effector genes may function as the real downstream genes for *TaDREB1* in response to these stresses.

The *TaDREB1* gene can trans-activate downstream genes in vivo in both yeast and transgenic *Arabidopsis*, indicating that it is a functional transcription factor. However, in different transgenic plants, *TaDREB1* exerted different effects. In transgenic rice, overexpression of the *TaDREB1* gene under an unstressed condition caused a dwarf phenotype. This phenotype was also observed in transgenic *Arabidopsis*. Whereas in *TaDREB1*-transgenic *Arabidopsis*, this dwarf phenotype was not observed. The discrepancy may result from the possibility that a gene from monocots only functioned effectively in its transgenic monocots, and a gene from dicots only functioned effectively in its transgenic dicots. If a gene from the monocot was transferred into dicots, it may not function effectively as it did in the monocot. Therefore, overexpresssion of the wheat *TaDREB1* caused a dwarf phenotype in rice, but not in Arabidopsis. Further studies on the transgenic plants will elucidate more details on the function of *TaDREB1*.

Acknowledgements The authors are grateful to Prof. Zhensheng Li for provide the seeds of wheat Xiaoyan 54. This work was supported by the National Transgenic Plants Program of China (J00-A-008-02), the Major Basic Research Program of China (G1999011703).

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