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ORIGINAL PAPER

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Soybean DRE-binding transcription factors that are responsive to abiotic stresses

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Abstract Three *DREB* homologue genes, *GmDREBa*, *GmDREBb*, and *GmDREBc*, were isolated from soybean, *Glycine max* (L.) Merr. Each of the deduced proteins contains an AP2 domain of 64 amino acids. Yeast one-hybrid assay revealed that all of the three dehydration-responsive, element-binding proteins specifically bound to the dehydration-responsive element. Analysis of transcriptional activation abilities of these proteins in yeast indicated that GmDREBa and GmDREBb could activate the expression of a reporter gene, whereas GmDREBc could not. The transcriptions of *GmDREBa* and *GmDREBb* were induced by salt, drought, and cold stresses in leaves of soybean seedlings. The expression of *GmDREBc* was not significantly affected in leaves but apparently induced in roots by salt, drought, and abscisic acid treatments. These results suggest that these three genes function specifically in response to abiotic stresses in soybean.

Introduction

Salt, drought, and low temperature induce expressions of many genes in various plants (Stockinger et al. 1997; Gilmour et al. 1998; Medina et al. 1999; Haake et al. 2002; Chinnusamy et al. 2003; Wang et al. 2003). The *cis*- and *trans*-acting elements play significant roles in stress-responsive gene expression. Yamaguchi-Shinozaki and Shinozaki (1994) identified a *cis*-acting dehydration-responsive element (DRE), which is present in the promoter of *COR78*/*RD29A* and involved in response to drought, low temperature, and high salt stresses. The *trans*-acting factor, DRE-binding (DREB), protein can bind to DRE to activate the gene expression in the stress-signaling pathway of plants. Many DREB proteins have been found in *Arabidopsis* (Liu et al. 1998; Medina et al. 1999). Liu et al. (1998) isolated two cDNA, *DREB1A* and *DREB2A*, encoding DREB proteins by using the yeast one-hybrid screening technique. The expression of the *DREB1A* gene was induced by low temperature, but the expression of the *DREB2A* gene was induced by dehydration. Recently, five cDNA for *DREB* homologues, *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, *OsDREB1D*, and *OsDREB2A*, have also been isolated from rice (Dubouzet et al. 2003). Each of the DREB proteins contains a conserved EREBP/AP2 domain of about 60 amino acids, which was first identified in the APETALA2 protein (Okamuro et al. 1997). The *APETALA2* gene plays a role in the control of *Arabidopsis* flower and seed development (Jofuku et al. 1994). The EREBP/AP2 domain has been found in many plant proteins encoded by regulatory genes, such as *Arabidopsis TINY* (Wilson et al. 1996), *DREBPs* (Liu et al. 1998; Chen et al. 2003; Shen et al. 2003a, 2003b), the *CBF* family (Medina et al. 1999), and Tobacco *Tsil* (Park et al. 2001).

Expression of *DREB* genes can be altered by members of the same DREB protein family. Novillo et al. (2004) reported that *Arabidopsis DREB1C*/*CBF2* was a negative regulator of *DREB1A*/*CBF3* and *DREB1B*/*CBF1* expression, although all three genes are responsive to abiotic stresses, indicating that their expressions are tightly controlled and thus guarantee the proper inductions of the downstream genes. The *DREB*/*CBF* genes can also be regulated by bHLH-type transcription factor, ICE1, which has been identified by ana-

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lyzing an *Arabidopsis ice1* mutant (Chinnusamy et al. 2003). The *DREB*/*CBF* gene expression may also be controlled by Ca^{2+}-related processes, because both mutations in the Ca^{2+}/H^{+} transporter CAX1 and Ca^{2+}-sensor protein CBL1 have altered patterns of *DREB*/*CBF* gene expressions (Albrecht et al. 2003; Catala et al. 2003).

Soybean is an important crop in the world. Isolation and characterization of stress-responsive genes from it may facilitate the generation of stress-tolerant soybean cultivars by genetic engineering approach. In the present study, we isolated three *DREB* genes from soybean and analyzed their DRE-binding activities. We also examined the transcriptional activation activities, genomic organizations, and expression patterns of these three genes under different stress conditions.

Materials and methods

Plant materials and stress treatments

Seedlings of soybean were grown in pots filled with vermiculite at 25°C under continuous light for 20 days, irrigated with water when necessary. The stress treatments were performed as follows: For salt, dehydration, and abscisic acid (ABA) treatments, we dipped the root of seedlings into the solutions of 250 mM NaCl, 20% PEG, and 100 μM ABA, respectively. For chilling treatment, seedlings were put into a 4°C growth chamber. All of four treatments were performed under constant illumination from cool-white fluorescent lights for various periods, and then the samples were harvested and frozen in liquid nitrogen for further analyses.

Gene cloning

An EREBP/AP2 consensus sequence was used as a query to search the expressed sequence tag (EST) database of soybean. The obtained ESTs were assembled and analyzed (Tian et al. 2004). Three sequences with highest homology to *Arabidopsis DREB* genes were selected and cloned by PCR using soybean genomic DNA as template. The primer sequences are as follows: for *GmDREBa*, 5′-GCAAAGTTTCCAAATTGAATG-3′ (forward) and 5′-AGTCATCTATCATGCAAGGC-3′ (reverse); for *GmDREBb*, 5′-GTTTTGGAATTGAGACAGGC-3′ (forward) and 5′-ACCAACCATTTGACATAACG-3′ (reverse); for *GmDREBc*, 5′-GTGTTTGTTGGAAGAGCTC-3′ (forward) and 5′-CAAAAGAATTGACCATTTTTG-3′ (reverse). PCR was performed as follows: for *GmDREBa*, 35 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min 20 s; for *GmDREBb*, 35 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min 30 s; and for *GmDREBc*, 35 cycles at 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min 20 s.

DRE-binding activities of the GmDREB proteins

To analyze DRE-binding activity, each of the full-length coding regions of *GmDREBs* and the *Arabidopsis AtDREB1* (as a positive control) was cloned into the *Eco*RI–*Xho*I sites of the GAL4 activation-domain vector (pAD). Then, the plasmids were transformed into yeast strain YM4271 carrying the reporter genes *His3* and *LacZ* under the control of *rd29A* promoter containing the DRE sequence (TACCGACAT) or mutated DRE (mDRE) sequence (TATTTTCAT), respectively (Liu et al. 1998). The growth status of the transformed yeast cells was compared on selective medium of synthetic dextrose (SD) medium without His plus 10 mM 3-aminotriazole (SD/−His + 10 mM 3-AT). The β-galactosidase activity was assayed as described (Chen et al. 2003).

The transcriptional activation activities of GmDREB proteins

Each of the three coding regions of *GmDREBa*, *GmDREBb*, and *GmDREBc* was cloned into the yeast expression vector (pBD) containing the binding domain (BD) of *GAL4*. Each of the plasmids was transformed into the yeast strain YRG-2, containing the reporter gene *His3*. If the encoded proteins possessed activation ability, it would work together with the BD of *GAL4* to promote the expression of the reporter gene *His3*, resulting in the growth of the transformed yeast cells on SD/−His + 10 mM 3-AT medium. Yeast cells containing pGAL4 and pBD were used as positive and negative controls, respectively.

DNA and RNA gel blot analysis

DNA extraction was performed as described previously (Chen et al. 1991). To prepare the DNA gel blots, genomic DNA (about 10 μg) was digested completely with 50 U of restrictive enzymes *Bam*HI, *Dra*I, *Eco*RI, and *Hin*dIII for 16–18 h, respectively, fractionated on a 0.8% agarose gel, and then blotted onto the Hybond-N^{+} nylon membrane in 0.4 M NaOH. The hybridization was performed as described (Chen et al. 2003), and the membrane was washed under a high-stringency condition.

Total RNA was extracted from soybean samples as described by Zhang et al. (1996). About 30 μg of total RNA was fractionated in a 1.2% agarose gel containing formaldehyde and then transferred onto Hybond-N^{+} nylon membrane in 20× SSC. The pre-hybridization was performed at 65°C for 4 h, and then hybridization was performed at 65°C for 18 h. The membrane was washed in 2× SSC, 0.1% SDS at 45°C for 15 min and in 1× SSC, 0.1% SDS at 45°C for 5 min. Then the membrane was autoradiographed by a phosphorimaging system. The probe was labeled with [α-^{32}P] dCTP by random priming. The RNA hybridizations using two independent sets

of RNA samples were performed, with identical results. Results from one experiment are presented.

RT-PCR analysis

Total RNA was extracted from different tissues of adult soybean plants grown in normal conditions or from roots of seedlings exposed to 250 mM NaCl, 20% PEG, 4°C, and 100 μM ABA for various periods, respectively. About 5 μg of total RNA was reverse-transcripted into cDNA at 37°C for 1 h. The primers and PCR conditions were identical to that of "gene cloning," except that 29 cycles were used. The *tubulin* gene was amplified as a control. The RT-PCR was performed using two independent sets of RNA samples, and the results are identical. Results from one experiment were presented.

Results

Isolation and characterization of *GmDREB* genes from soybean

To isolate the genes encoding the DRE-binding protein from soybean, an EREBP/AP2 consensus sequence was used for a BLAST search against the EST database of soybean, and 163 unigenes were obtained after assembly (Tian et al. 2004). These unigenes were further subject to homology analysis using the BLAST program against the sequences from the NCBI database. Three sequences, with high homology to the *Arabidopsis DREB* genes, were selected and cloned by PCR from soybean genomic DNA. Although cloned from genomic DNA, the three sequences were identical to the three assembled EST sequences. The corresponding genes were named as *GmDREBa* (GenBank accession no. AY542886), *GmDREBb* (GenBank accession no. AY296651), and *GmDREBc* (GenBank accession no. AY244760), respectively. Sequence analysis reveals that they all contain full-length open-reading frames, and there are no introns in their coding regions.

GmDREBa, *GmDREBb*, and *GmDREBc* encode polypeptides of 211, 312, and 198 amino acids with predicted molecular mass of 23, 35, and 21 kDa, respectively (Fig. 1a). Further analysis of the deduced amino acids (using SMART program: http://smart.embl-heidelberg.de) revealed that each protein contains a conserved DNA binding domain (AP2 domain) of 64 amino acids (Fig. 1a).

We compared the deduced amino acid sequences of the three *GmDREB* genes with other known DREB proteins. As shown in Fig. 1a, among the three GmDREB proteins, GmDREBa, and GmDREBc are closely related, with 91% identity in the AP2 domain. The identities of AP2 domain between GmDREBa and GmDREBb and between GmDREBb and GmDREBc are both 64%. The three proteins show less identity outside the AP2 domain or when compared with the proteins listed. Overall, GmDREBa had 72% similarity and 71% identity when compared with GmDREBc, and showed 30% similarity and 24% identity in comparison with GmDREBb. GmDREBc exhibited 32% similarity and 25% identity when compared with GmDREBb. Phylogenetic analysis (Fig. 1b) shows that GmDREBa and GmDREBc are clustered with AtDREB2A (AB016570), whereas, GmDREBb may be more related to GmDREB1 (AF514908) and AtDREB1A (NM118680). GmDREB1 is another DREB-type transcription factor gene from soybean. Its characterization has not been reported.

DRE-binding activities of the three GmDREB proteins

To analyze the DRE-binding activities of GmDREBa, GmDREBb and GmDREBc, the entire coding region of each gene was fused in-frame with the GAL4 activation-domain in a pAD vector, and the obtained plasmids were transformed into two yeast strains carrying the dual reporter genes *His3* and *LacZ* under the control of DRE or mDRE motif, respectively. The *Arabidopsis AtDREB1* gene was done similarly and used as a positive (for DRE) or a negative (for mDRE) control, respectively (Fig. 2a). The growth status of these transformed yeast cells was analyzed. The results (Fig. 2b) showed that the yeast cells harboring each of the GmDREBs and the DRE-controlled reporter genes could grow well on selective medium of SD/−His + 10 mM 3-AT, and colonies turned blue when *LacZ* activity was examined with X-gal. However, the transformed yeast cells harboring each of the GmDREBs and the mDRE-controlled reporter genes could not grow in the same medium. These results indicate that GmDREBa, GmDREBb, and GmDREBc can bind to DRE but not the mDRE element.

Analysis of the transcriptional activation ability of the GmDREBs

We next analyzed the in vivo transcriptional activation abilities of GmDREBs. The coding regions of *GmDREBa*, *GmDREBb*, and *GmDREBc* were subcloned into pBD containing the DNA-BD of *GAL4*. Each of the plasmids was transformed into the yeast strain YRG-2 containing the reporter gene *His3*. If the GmDREB proteins possess transcriptional activation abilities, they would cooperate with the BD of GAL4 to promote the expression of *His3* gene, resulting in the well growth of the transformed yeast cells on SD/−His + 10 mM 3-AT (Fig. 3a). Figure 3b shows that the transformed yeast cells harboring pBD-GmDREBa, pBD-GmDREBb, pBD-GmDREBc, pGAL4 (positive control), and pBD (negative control) all could grow on YPAD medium. However, only the yeast cells containing pBD-GmDREBa, pBD-GmDREBb, and pGAL4 could grow on SD/−His + 10 mM 3-AT. These results

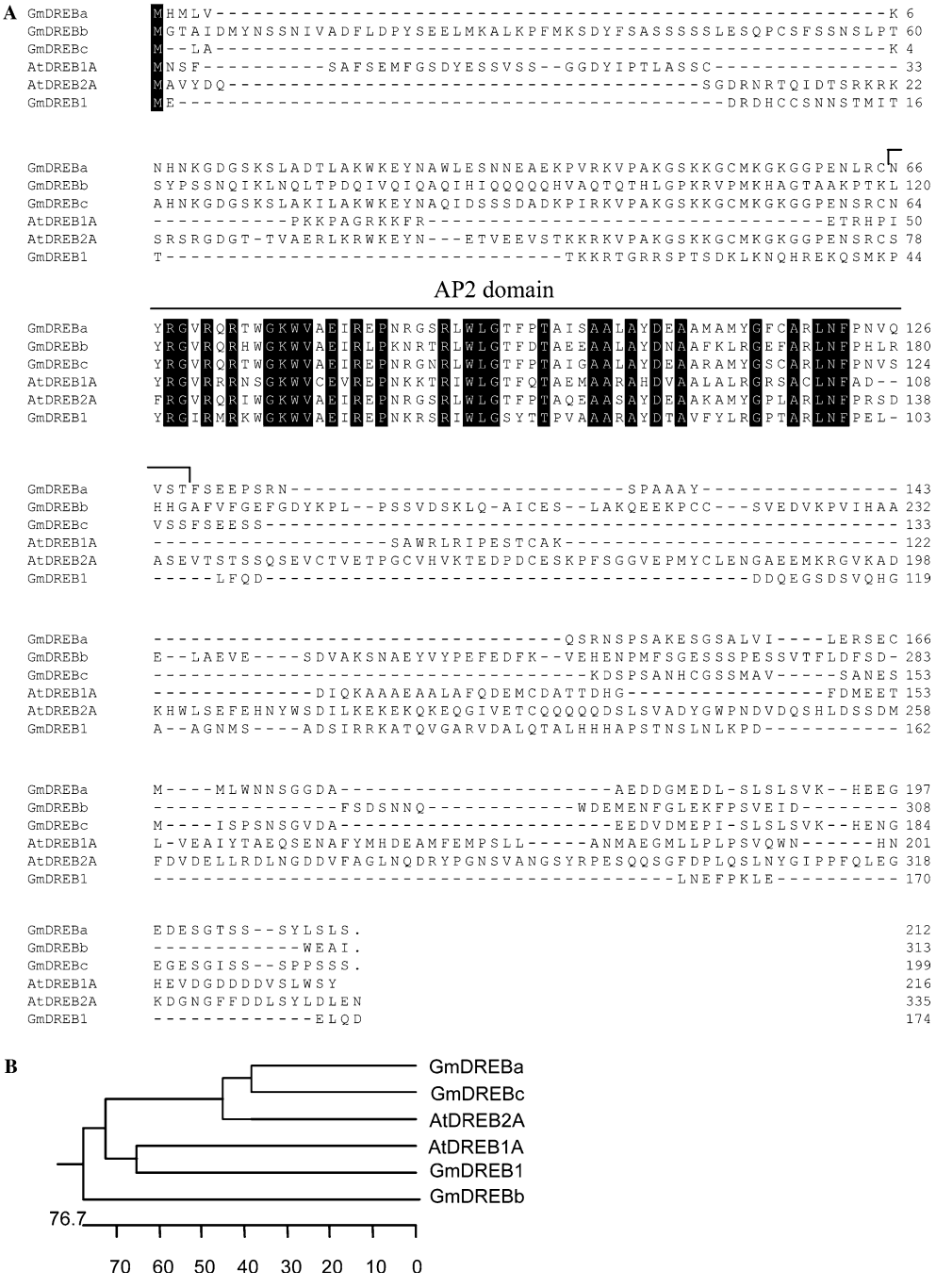

Fig. 1 Comparison and phylogenetic analysis of the deduced amino acid sequences of GmDREBa, GmDREBb, and GmDREBc. **a** Comparison of deduced amino acids of GmDREBa, GmDREBb, and GmDREBc with other EREBP/AP2 proteins. The *shades* indicate identity. AtDREB1A (NM118680) and AtDREB2A (AB016570) are from *Arabidopsis*. GmDREB1 (AF514908) is from soybean. **b** Phylogenetic tree shows the relationships of the six proteins. The neighbor-joining tree is based on an alignment of the complete protein sequences. Bootstrap values are shown at the *bottom*

indicate that GmDREBa and GmDREBb could activate the transcription of the reporter gene, whereas GmDREBc protein could not.

DNA gel blot analysis

The genomic organizations of *GmDREBa* and *GmDREBb* in soybean genome were examined by Southern analysis. Genomic DNA of soybean was digested completely with *Bam*HI, *Dra*I, *Eco*RI, and *Hin*dIII, respectively. After separation on agarose gel, the DNA was transferred onto membrane and hybridized with ^{32}P-dCTP-labeled full-length cDNA of *GmDREBa* and *GmDREBb*, respectively. Figure 4a showed that, for *GmDERBa* gene, there were one strong hybridization band and one weak band in both *Bam*HI and *Hin*dIII digestions. However, there was only one band in *Dra*I and *Eco*RI digestions. Considering no restriction sites of the four enzymes in *GmDREBa* sequence, it is likely that only one copy of *GmDREBa* is present in the soybean genome. In Fig. 4b, one to three bands in each lane were observed for *GmDREBb* gene. Because one *Dra*I site and one *Hin*dIII site are found in the *GmDREBb* sequence, we presume that *GmDREBb* is also a single-copy gene in the genome.

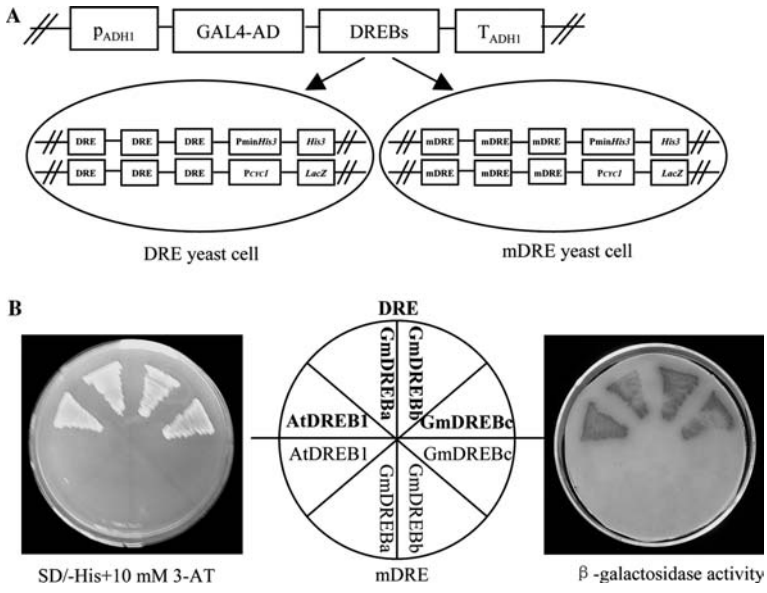

Fig. 2 The dehydration-responsive element (*DRE*)-binding (*DREB*) specificity analysis. **a** Construction of pAD-GmDREBs plasmids. Each of the coding regions of *GmDREBa*, *GmDREBb*, and *GmDREBc* was fused to the activation domain of GAL4, and then the plasmids were transformed into yeast cells harboring DRE-controlled or mDRE-controlled reporter genes, respectively. P_{ADH1} is the promoter of the *ADH1* gene; T_{ADH1} is the terminator of the *ADH1* gene. **b** The transformed yeast cells were examined for growth on synthetic dextrose (SD) medium without His plus 10 mM 3-aminotriazole (SD/-His + 10 mM 3-AT) at 30°C (*left panel*) and tested for *β*-galactosidase activity (*right panel*). The expression of *AtDREB1* was used as a control. The *middle panel* shows the position of each transformed yeast cell, the *upper part* indicates yeast cells harboring DREB proteins and DRE-controlled reporter genes, and the *lower part* indicates yeast cells harboring DREB proteins and mDRE-controlled reporter genes

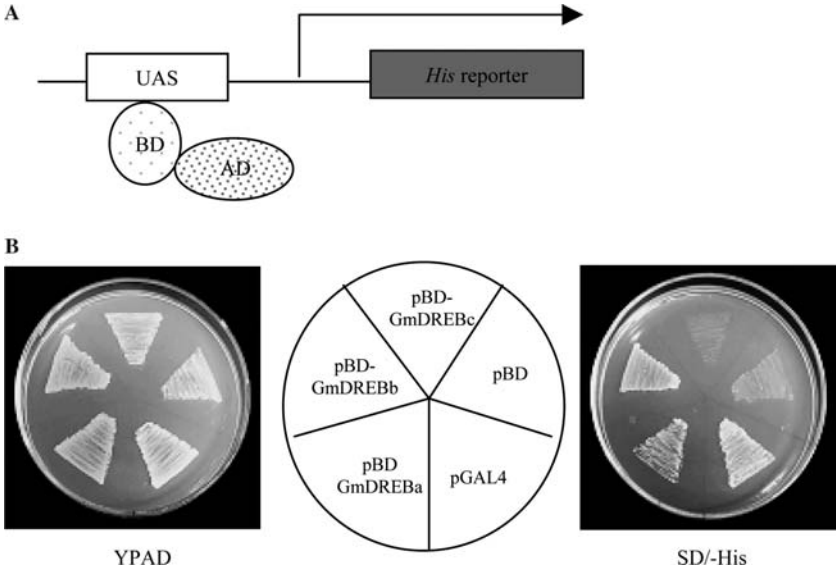

Fig. 3 Analysis of the transcriptional activation ability. **a** A sketch map of *His* reporter gene expression. *UAS* indicates upstream activating sequences, *AD* indicates the activation domain of the GmDREBs, and *BD* indicates the binding domain of GAL4 encoded in yeast expression vector (*pBD*) vector. *Arrowhead* indicates the direction of *His* gene expression. **b** Plasmids of pBD-GmDREBa, pBD-GmDREBb, pBD-GmDREBc, pGAL4 (positive control), and pBD (negative control) were transformed into yeast strain YRG-2, respectively. The transformed yeast cells were incubated on YPAD (*left panel*) or on SD/−His + 10 mM 3-AT (*right panel*) at 30°C for 3 days to examine their growth status. The *middle panel* indicates the position of each yeast strain

Expression patterns of the three *GmDREB* genes under various treatments

Northern analysis was performed to investigate the expression patterns of *GmDREBa*, *GmDREBb*, and *GmDREBc* under various treatments. Total RNA was extracted from leaves of soybean seedlings of 20 days treated with 250 mM NaCl, 20% PEG, 4°C, and 100 μM ABA in different time points, respectively. As shown in Fig. 5a, *GmDREBa* and *GmDREBb* had sim-

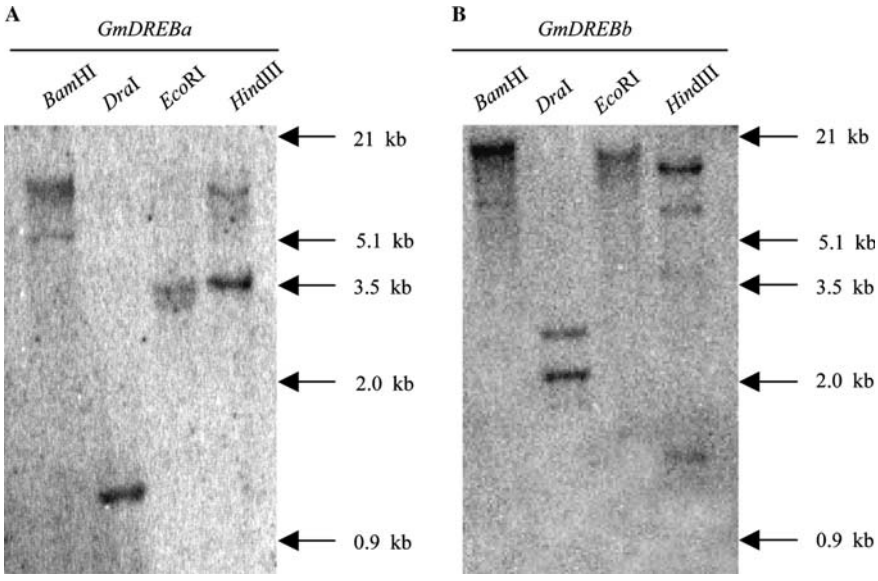

Fig. 4 Southern blot analysis of *GmDREBa* and *GmDREBb* genes. The full-length cDNA of *GmDREBa* and *GmDREBb* was labeled and used as probes to hybridize with genomic DNA digested by *Bam*HI, *Dra*I, *Eco*RI, or *Hin*dIII, respectively. **a** Southern blot analysis of *GmDREBa* gene. **b** Southern blot analysis of *GmDREBb* gene. *Numbers on the right of each panel* indicate the size of DNA markers

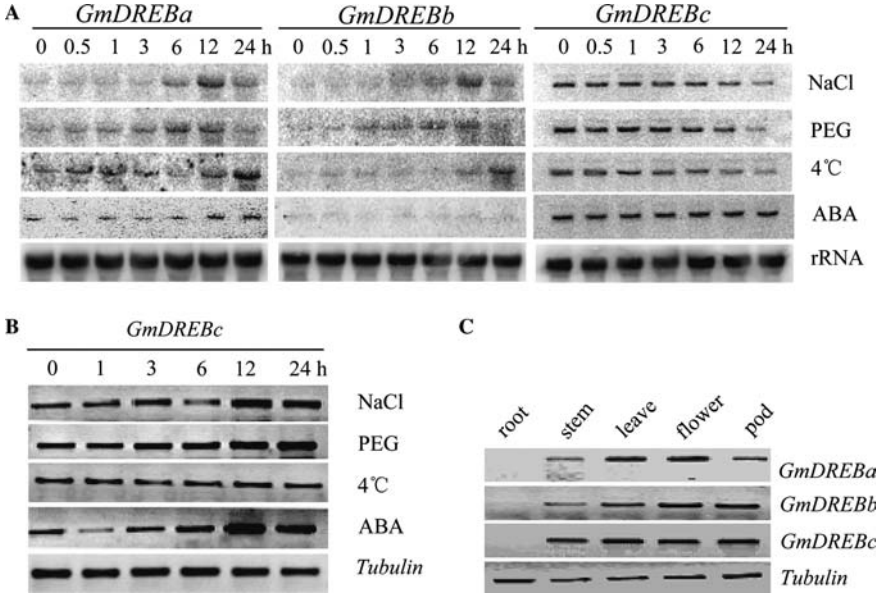

Fig. 5 Expression patterns of *GmDREBa*, *GmDREBb*, and *GmDREBc*. **a** Expression patterns of the three *GmDREB* genes in response to various treatments. Northern analysis was performed with total RNA (30 μg) isolated from leaves of soybean seedlings exposed to 250 mM NaCl, 20% PEG, low temperature (4°C), and 100 μM abscisic acid (*ABA*) for the indicated time. The entire coding regions of the three *GmDREB* genes were used as probe, respectively. The rRNA hybridization was performed to check the RNA loading. Only the rRNA hybridization results for the NaCl-treated samples were presented. The rRNA hybridizations for other treatments also demonstrated equal loading and were omitted for simplicity. **b** Expression pattern of *GmDREBc* in roots of soybean seedlings. The root of soybean seedlings was treated as in **a** and sampled to extract total RNA. Five micrograms of total RNA were reverse-transcripted into first-strand cDNA for RT-PCR. **b** The *tubulin* gene was amplified as a control. The *tubulin* gene amplification results for NaCl-treated samples were presented. The amplifications of the *tubulin* gene for other treatments also proved the equal expression and were omitted for simplicity. **c** Tissue-specific expression of the three *GmDREB* genes. Total RNA (5 μg) isolated from roots, stems, leaves, flowers, and pods of the adult plants were reverse-transcripted into first-strand cDNA in a 25-μl reaction volume, and the cDNA was subject to RT-PCR. The *tubulin* gene was amplified as a control

ilar expression patterns under salt, drought, and cold treatments. Both of them had a low expression level under the normal conditions. Upon salt treatment, their mRNA accumulated at 6 h after the initiation of the treatment and reached maximum at 12 h. At 24 h, the mRNA levels were decreased to a low level. For drought treatment, the transcripts of *GmDREBa* and *GmDREBb* were induced earlier than the salt treatment, and reached maximum at 6–12 h. Under low-temperature conditions, the transcripts of both genes accumulated to highest levels at 24 h. The *GmDREBa* gene was slightly induced by ABA, but the expression of *GmDREBb* was not significantly affected by ABA treatment and was kept in a very low level.

We also investigated the expression of the *GmDREBc* gene and found that this gene was not affected by salt, drought, low temperature, and ABA in leaves of soybean seedlings (Fig. 5a). We isolated total RNA from roots of soybean seedlings and analyzed the expression pattern of *GmDREBc* using RT-PCR, because its expression in roots is too low to be detected by Northern blots. As shown in Fig. 5b, in roots, the expression of *GmDREBc* was induced by salt, drought, and ABA treatments, but not affected by low temperature.

The tissue-specific expression patterns of the three *GmDREB* genes were also examined by RT-PCR using total RNA isolated from roots, stems, leaves, flowers, and pods of adult soybean plants. Figure 5c shows that the transcripts of all of the three *GmDREB* genes were undetectable in roots. However, they had expressions in all the other organs tested.

Discussion

DREB-type transcription factor genes have been found in various plants, such as *AtDREB* (1A, 1B, 1C, 2A, 2B) from *Arabidopsis* (Liu et al. 1998; Nakashima et al. 2000), *OsDREB* (1A, 1B, 1C, 1D, 2A) from rice (Dubouzet et al. 2003), *AhDREB1* from *Atriplex hortensis* (Shen et al. 2003a), and *TaDREB1* from wheat (Shen et al. 2003b). However, their homologues in soybean have not been investigated. We cloned three *DREB* genes from soybean and named *GmDREBa*, *GmDREBb*, and *GmDREBc*, respectively. Each gene encodes a protein with a conserved AP2 domain of 64 amino acids, which is important to DREB proteins. In AP2 domain, the 14th valine (V) and the 19th glutamic acid (E) may be decisive for DNA-binding specificity of DREB proteins (Sakuma et al. 2002). Dubouzet et al. (2003) reported that OsDREB1A protein containing a valine at the 14th position but not glutamic acid at the 19th position had a more-efficient binding to GCCGAC than to ACCGAC (DRE core motif). However, OsDREB2A protein containing a valine at the 14th position and a glutamic acid at the 19th position bound to GCCGAC and ACCGAC at the same efficiency. In the present study, both GmDREBa and GmDREBc proteins have a conserved valine at the 14th position and glutamic acid at the 19th position, whereas GmDREBb protein has a valine at the 14th position and a leucine at the 19th position. DRE-binding test demonstrates that all the three proteins can bind to the DRE element. However, whether they have any affinity to GCCGAC motif remains to be tested.

We examined transcriptional activation abilities of GmDREBa, GmDREBb and GmDREBc. The GmDREBa and GmDREBb proteins could interact with the binding domain of GAL4 to activate the expression of the reporter gene but the GmDREBc protein could not. It is possible that the GmDREBc protein activate the expression of its downstream genes in plants by interacting with other proteins. Alternatively, it may function as a transcriptional repressor. Therefore, although all of the three proteins can bind to the DRE motif, they may have different functions in regulating the expressions of their downstream genes. Transcriptional activators and repressors have been found in the subfamily of the ethylene-responsive element binding factors in *Arabidopsis* (Fujimoto et al. 2000).

The three *GmDREB* genes in adult soybean plants showed slight different expression level in stems, leaves, flowers, and pods, and no expression was detected in roots. These results suggest that the three *GmDREB* genes may mainly function in the aerial parts of adult soybean plants.

Expressions of both *GmDREBa* and *GmDREBb* were induced by salt, drought, and low temperature in leaves of soybean seedlings. Their similar expression patterns indicate that these two genes may function similarly in some degree in plant responses to abiotic stresses. *GmDREBc* was not induced by salt, drought, low temperature, or ABA in leaves of soybean seedlings. However, in roots of young seedlings, *GmDREBc* was induced by salt, drought, and ABA treatments. This implies that the *GmDREBc* gene is more responsive to unfavorable environment in roots than in leaves. This is reasonable, because roots usually sensed the stress signals from the soil in the earliest time, so the plants need to express some genes in roots in time to help it survive severe conditions. Previously, Shen et al. (2003a) also found that *AhDREB1*, a *DREB*-type gene from *A. hortensis*, was induced in roots, but not in stems and leaves. It should be mentioned that although *GmDREBc* is expressed in roots of soybean seedlings (Fig. 5b), it could not be detectable in roots of adult plants (Fig. 5c). This phenomenon may reflect the subtle difference of *GmDREBc* functions at different developmental stages.

It is interesting to note that although GmDREBa is more similar to GmDREBc than to GmDREBb in amino acid sequence, its transcriptional activation ability and stress-induction pattern resemble those of GmDREBb. It is possible that expressions of *GmDREBa* and *GmDREBb* are controlled by the same factor and the two proteins formed a heterodimer to coordinately regulate downstream gene expression. However, whether this is the case needs to be confirmed. For *GmDREBc*, its expression was constitutive in leaves and stress-regulated in young roots, indicating the presence of a differential regulatory mechanism. The *GmDREBc* may play roles in more general processes in leaves and function specifically in roots during stress responses. It is also possible that the three genes regulate each other's expression, because in *Arabidopsis*, *DREB1C* has been found to regulate *DREB1A* and *DREB1B* (Novillo et al. 2004).

Different expression patterns of the *GmDREBa*, *GmDREBb*, and *GmDREBc* genes and different transcriptional activation ability of the corresponding proteins imply that they may exert different effects on plants when exposed to disadvantageous environment. Transformation of the *GmDREBa*, *GmDREBb* and *GmDREBc* genes into *Arabidopsis* and further analysis

should reveal their possible functions in plant abiotic stress tolerance.

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