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RESEARCH ARTICLE

Cloning and functional identification of stress-resistant *BeDREB* genes from Bermuda grass

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Abstract Dehydration-responsive element-binding (DREB) proteins, specifically binding to the dehydration-responsive element (DRE), have been identified as a group of important transcription activators of plants which regulate the expression of genes in response to drought, high-salt and low- temperature stresses. Two DREB-like genes from Bermuda grass that are induced by low-temperature or high-salt stresses were cloned using RT-PCR and RACE methods, and were named *BeDREB1* and *BeDREB2*, respectively (GenBank accession No: AY462117 and AY462118). They contained an ORF of 753 bp encoding 251 amino acids, showing the typical characteristics of the DREB gene family. Interestingly, these two genes isolated from Bermuda grass induced either by low-temperature stress or high-salt stress shared 97.8% homology. Furthermore, it was demonstrated that both BeDREB1 and BeDREB2 could bind to the wild-type DRE element to activate the transcription of the reporter gene *HIS3*, driven by a promoter carrying DRE *cis*-element in yeast strain 4721, in the presence of 3-AT. RT-PCR showed that *BeDREB1* and *BeDREB2* genes could be greatly induced by low-temperature and high-salt stresses, respectively. Their expressions were changed following the inducible time. In conclusion, all results indicate that *BeDREB1* and *BeDREB2* genes isolated from treated Bermuda grass are new members of the DREB transcription activator family, which may play very important roles in signal transduction related to stresses.

Keywords Bermuda grass, *BeDREB*, DRE element, molecular cloning, transcription activation

1 Introduction

Drought, high-salt and low-temperature stresses are important factors affecting the development of plants and the yields of crops. When exposed to these abiotic stresses, plants will undergo a series of physiological and biochemical changes, which lead to the activation or inactivation of some specific endocellular signaling pathways in response to stimuli (Shinozaki and Yamaguchi-Shinozaki, 1996, Liu et al., 1998). There are mainly two sorts of genes involved in these processes. Some encode proteins that can either participate in protecting plant cells from the stresses or repair injuries caused by the stresses, and the others are the protein kinases that respond to and transduct the stress-induced signals or transcription activators (Shinozaki and Yamaguchi-Shinozaki, 1996). Yamaguchi Shinozaki and his colleagues cloned a batch of drought-induced genes from drought-treated Arabidopsis named *rd* (responsive dehydration) genes, using the method of differentiation display. Among them, the expression of *rd29A* is induced by drought, high-salt and low-temperature conditions and ABA. In addition to containing the ABRE element, *rd29A* promoter also has two ATCCGACTA-containing *cis*-DREs (dehydration-responsive element) that respond to ABA-independent drought stress (Yamaguchi-Shinozaki et al., 1992; Yamaguchi-Shinozaki and Shinozaki, 1994; Shinozaki and Yamaguchi-Shinozaki, 1996; Shinozaki and Yamaguchi-Shinozaki, 2000). Furthermore, many genes that respond to drought, high-salt and low-temperature conditions were discovered to have DRE or CRT (C-repeat element) in their promoter regions (Yamaguchi-Shinozaki and Shinozaki , 1994; Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000). Liu et al. (1998) obtained five transcription activators, i.e. DREB1 A-C and DREB2 A-B, by screening the Arabidopsis cDNA library and by using the yeast one-hybrid method, which specifically bind to DRE and regulate the expression of stress-resistant genes. DREB1s are induced by low-temperature, whereas DREB2

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is induced by drought and high-salt conditions. DREB1 and DREB2 independently function as transcription activators in low-temperature and drought-induced signaling pathways, respectively.

Stress-resistance in plants is usually controlled by multiple functional genes instead of a single or few genes. A transcription activator is capable of regulating the expression of several genes. Thus, the enhancement of a stress-resistant transcription activator will lead to the expression of several functional stress-resistant genes. This is an effective way to comprehensively improve the properties of plants (Kizis et al., 2000; Liu et al., 2000). *Cis*-DRE (or DRE core sequence element) universally exists in the promoters of the genes related to drought, high-salt and low- temperature responses. DREB transcription activators activate the expression of several downstream genes by binding the cis-DRE, which consequently enhances the stress resistance of plants (Yamaguchi-Shinozaki and Shinozaki, 1994; Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000).

Recently, the lawn industry has become a rapidly developing industry and plays very important roles in playing field construction, city beautification and soil conservation. However, the lawn industry consumes large amounts of water and is one of the most environmentally susceptible industries. Enhancement of the resistance of grass to low temperatures, drought and salt conditions is the most pressing problem. *DREB* genes have been discovered in many kinds of plants, including *Arabidopisis thaliana, Nicotiana tabacum, Triticum aestivum, Oryza sativa, Gossypium herbaceum and Zea mays* (Riechmann and Meyerowitz, 1998; Riechmann et al., 2000; Qin et al., 2003), but none has been reported in lawn grass. Being a qualified warm-season grass, Bermuda grass is highly elastic and resistant to trampling, and thus is used as the main species in constructing football and golf courses. Here, we reported two DREB-like genes, *BeDREB1* and *BeDREB2*, identified from Bermuda grass. Yeast one-hybrid screening demonstrated that *BeDREB* proteins can activate the transcription of downstream genes by binding downstream DRE.

2 Materials and methods

2.1 Plant materials, strains, vectors and reagents

Bermuda grass (*Cynodon dactylon*) seeds were kindly provided by the Institute of Genetics and Developmental Biology (IGDB) of the Chinese Academy of Sciences. Cloning vectors pGEM-T Easy vectors and *Taq* enzymes were purchased from Promega. Restriction enzymes, T4 DNA ligase, cDNA Synthesis Kit, 3′-Full RACE Core Set and RT Real Time One Step PCR Kit were purchased from TaKaRa. TRIzol reagent and 5′RACE System for Rapid Amplification of cDNA Ends (Version 2.0) were purchased from Invitrogen. Yeast YEP culture medium, SD-deficient culture medium and yeast competent cell preparation reagents were purchased from Clontech.

2.2 Plant material treatment

The seeds were soaked overnight, sterilized for 12 min by 0.1% $HgCl_2$ and washed 4–5 times. These seeds were then planted in 0.8% agar medium containing 1/2 MS inorganic salt and cultivated in light 12 h/d after incubation in the dark at 26ºC for germination. The seedlings were subjected to treatment 3 weeks later. For low-temperature and high-salt treatments, the seedlings were placed at 4ºC for 5 h, or soaked in 250 mmol/L NaCl solution for 5 h to induce the related gene expression. After treatment, the seedlings were washed and frozen at –80ºC for further use.

For expression analysis, the seedlings were kept at 4ºC or in high-salt solution (250 mmol/L NaCl) for 1, 4, 8 and 12 h, respectively and the untreated seedlings used as control. The total RNA was then extracted from the treated samples using TRIzol reagents.

2.3 Amplification of *BeDREB*

Degenerate primers were designed according to the DNA sequence of the AP2/EREBP conserved domains of known DREB transcription activators. The forward and backward primer sequences were AP2 pair as indicated in Table 1. RT-PCR was carried out with the synthesized cDNA from total RNA of low-temperature and high-salt treated samples as the template and AP2 as primers. The PCR conditions were ① 94ºC 2 min, ② 94ºC 30 s, 50ºC 30 s, 72ºC 30 s, 30 cycles, ③ 72ºC 10 min. The amplified products were sequenced. Based on the amplified conservative sequences, two forward primers, 3race1 and 3race2, for 3’ end amplification were synthesized. Together with Oligo dT, nesting PCR was performed. The same primers were used for the two different genes because of their high similarity. The forward primer for the 5’ end cDNA amplification was the adapter provided in the kit. The reverse transcription primers and PCR reverse primers were designed according to the conservative regions of the two cDNAs and 3’ cDNA joining segments, respectively. The reverse transcription primer of the low-temperature and high-salt treated mRNA is Zrt, indicated in Table 1. The two reverse nesting primer sequences for low-temperature induced cDNA 5’ end amplification were Na-5race1 and Na-5race2 (Table 1). The amplification conditions were ① 94ºC, ② 94ºC 45 s, 55ºC 45 s, 72ºC 50 s, 30 cycles, ③ 72ºC 10 min. The amplified products were verified by sequencing and the overlapped regions of the segments were joined to form the full length. For amplifying the full length of the gene, the primers were designed according to the joined segments. Forward and backward primers for amplifying the low-temperature and high-salt inducing genes were Co-full and Na-full primer pairs, respectively (Table 1). PCR amplification was performed using the cDNA of treated samples as the template. The target fragments were cloned into pGEM-T-Easy vector and sequenced.

Table 1 Primers used in amplifying *BeDREB* genes

Primers	Sense Primers (5′ to 3′)	Anti-sense Primers (5′ to 3′)
AP2	AA(G)TGGGTT(G)GCTGAGATCCGTG	GCGCCATACATTGCCCTTGC
3race1	ATGGCTTGGTTCATTCCC	oligo dT Adaptor Primer
3race2	TGGTTCATTCCCTACCGC	oligo dT Adaptor Primer
Zrt	AGTCTCCACAGATTCCACCT	
Co-5race1	Adaptor Primer	TCATCAGACCGTTGAGAAGC
Co-5race2	Adaptor Primer	TTGCCACGGTTGGGCTCACG
Na-5raceR1	Adaptor Primer	GTATGTTAGACGCCAGCAAG
Na-5raceR2	Adaptor Primer	TTGCCACGGTTGGGCTCACG
Co-full	TCGGACCAGTTGGAGGAAAT	CGCGGTTGGCTTATAGTATAGAG
Na-full	AGACCAGTTGGAGGAAAT	TTGGGTTCAACGAGTAAA
Actin	CTTAACCCTAAGGCTAACAG	TCCTCCGATCCAGACACT

2.4 RT-PCR analysis of the *BeDREB* gene expression in low-temperature and high-salt conditions

Total RNA was extracted from the Bermuda grass seedlings treated using low-temperature or high-salt conditions following the time course described in Section 2.2. The absorbance of total RNA at 260 nm was measured by a spectrometer. RT-PCR analysis was performed using the same amount of total RNA obtained from different treatments as the template to examine *BeDREB1* and *BeDREB2* expression. The primers were Co-full and Na-full pairs (Table 1). Bermuda actin was used as the control.

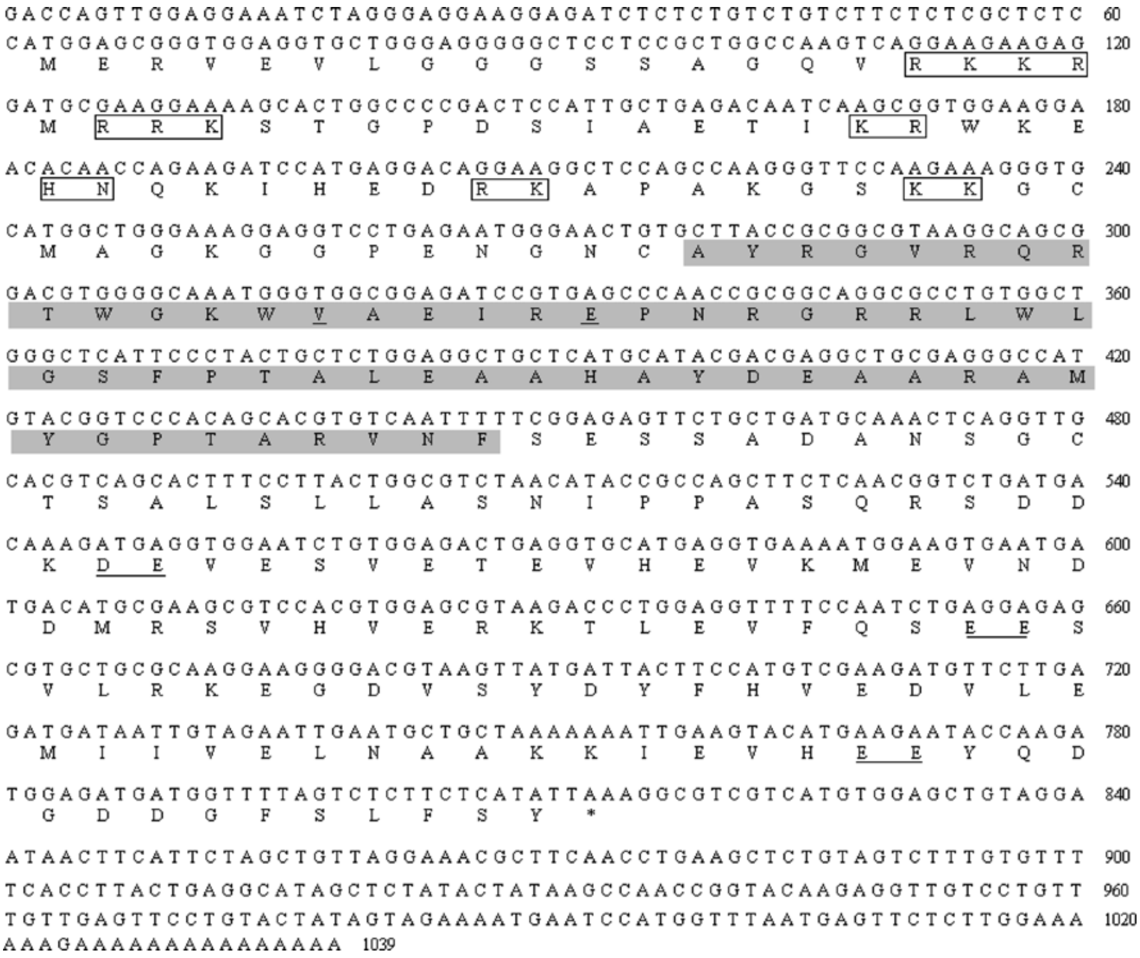
2.5 Yeast one-hybrid analysis of *BeDREB* transcription activity

BeDREB1 and *BeDREB2* transcription activity was analyzed using yeast one-hybrid system. The yeast strain, YW4721, contained double reporter genes (*lacZ* and *HIS3*) driven by the 35s strong promoter, which had a three-tandem DRE upstream. The full-length *BeDREB* genes were cloned into YepGAP expression vectors to form YepGAP-*BeDREB*1 and YepGAP-*BeDREB*2 plasmids. These plasmids were transformed into wild type (wt) and mutant (md) yeast YW4271 strains, respectively, for analyzing the transcription activation. Reporter genes in the wild-type strain can be activated by DREB as it contains *cis*-DRE upstream, whereas the mutant cannot be activated because of the mutated *cis*-DRE that could no longer be activated by DREB. YepGAP-DREB1A vector was used as the positive control and YEP alone as the negative control. The transformants were inoculated in His$^-$/Trp$^-$/Ura$^-$-deficient SD plates containing different concentrations of 3-AT for observing the growth of the yeasts at 30ºC after 3–4 h.

3 Results

3.1 Sequence analysis of *BeDREB*

The full length of the *DREB*-like gene amplified from the low-temperature treated *Bermuda* seedlings by RT-PCR and RACE was 1,039 bp and its ORF (open reading frame) was 753 bp encoding 251 amino acid residues (Fig. 1). The full length of the *DREB*-like gene amplified from the high-salt treated seedlings was 1,061 bp with an ORF of 753 bp encoding 251 amino acid residues (Fig. 2). Both have been submitted to the NCBI GenBank database (Accession no. AY462117 and AY462118). Bioinformatic analysis indicated that the coding regions of both the genes contained a typical AP2/EREBP conserved region consisting of 57 amino acids, with high homology to those cloned from other plants. There were two functional amino acids, valine (V) and glutamate (E) in position 14 and 19, which have been proved to specifically bind to cis-DRE. Besides these, the two genes also possess other typical properties of DREB transcription activators, i.e. a basic amino acid-rich nucleus-localization signal region at the N-terminal and an acidic transcription activation region at the C-terminal. It was concluded that the two *DREB*-like genes play similar functional roles to those from other plants in regulating Bermuda grass responses to abiotic stresses and the expression activation of stress-resistant genes. Alignment indicated that the *DREB*-like genes in Bermuda grass shared high homology with those from different plant species, including TaDREB in *Triticum aestivum*, OsDREB in *Oryza sativa* and *DREB1* (A–C) and *DREB2* (A–B) in *Arabidopsis* (Fig. 3). *DREB*-like genes of Bermuda either treated by low-temperature or high-salt conditions shared 97.8% amino acid homology and have almost the same amino acid sequences in their conserved region. They were named *BeDREB1* and *BeDREB2*.



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GATGCGAAGGAAAAGCACTGGCCCCGACTCCATTGCTGAGACAATCAAGCGGTGGAAGGA 180
  M  R  R  K  S  T  G  P  D  S  I  A  E  T  I  K  R  W  K  E

ACACAACCAGAAGATCCATGAGGACAGGAAGGCTCCAGCCAAGGGTTCCAAGAAAGGGTG 240
  H  N  Q  K  I  H  E  D  R  K  A  P  A  K  G  S  K  K  G  C

CATGGCTGGGAAAGGAGGTCCTGAGAATGGGAACTGTGCTTACCGCGGCGTAAGGCAGCG 300
  M  A  G  K  G  G  P  E  N  G  N  C  A  Y  R  G  V  R  Q  R

GACGTGGGGCAAATGGGTGGCGGAGATCCGTGAGCCCAACCGCGGCAGGCGCCTGTGGCT 360
  T  W  G  K  W  V  A  E  I  R  E  P  N  R  G  R  R  L  W  L

GGGCTCATTCCCTACTGCTCTGGAGGCTGCTCATGCATACGACGAGGCTGCGAGGGCCAT 420
  G  S  F  P  T  A  L  E  A  A  H  A  Y  D  E  A  A  R  A  M

GTACGGTCCCACAGCACGTGTCAATTTTTCGGAGAGTTCTGCTGATGCAAACTCAGGTTG 480
  Y  G  P  T  A  R  V  N  F  S  E  S  S  A  D  A  N  S  G  C

CACGTCAGCACTTTCCTTACTGGCGTCTAACATACCGCCAGCTTCTCAACGGTCTGATGA 540
  T  S  A  L  S  L  L  A  S  N  I  P  P  A  S  Q  R  S  D  D

CAAAGATGAGGTGGAATCTGTGGAGACTGAGGTGCATGAGGTGAAAATGGAAGTGAATGA 600
  K  D  E  V  E  S  V  E  T  E  V  H  E  V  K  M  E  V  N  D

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  D  M  R  S  V  H  V  E  R  K  T  L  E  V  F  Q  S  E  E  S

CGTGCTGCGCAAGGAAGGGGACGTAAGTTATGATTACTTCCATGTCGAAGATGTTCTTGA 720
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TCACCTTACTGAGGCATAGCTCTATACTATAAGCCAACCGGTACAAGAGGTTGTCCTGTT 960
TGTTGAGTTCCTGTACTATAGTAGAAAATGAATCCATGGTTTAATGAGTTCTCTTGGAAA 1020
AAAGAAAAAAAAAAAAAAA 1039
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The AP2/EREBP domain was black background. The N-terminal region, which might function as a nuclear localization signal, was framed. There was an acidic C-terminal region, which might act as a transcriptional activation domain, and was underlined. The nucleotide sequence had been submitted to the NCBI GenBank (GenBank accession No: AY462117)

Fig. 1 Nucleotides and deduced amino acid sequence of the cDNA encoding BeDREB1 from *Bermuda grass* in cold stress

3.2 Expression pattern of *BeDREB*

The expression patterns of *BeDREB1* and *BeDREB2* were examined by RT-PCR after different treatment as described in Section 2.4 (Fig. 4). The result showed that *BeDREB1* and *BeDREB2* are expressed differently (Fig. 4), but both *BeDREB1* and *BeDREB2* showed higher amplification levels after treatment for 1 h. The amplification level went up following the inducible time and reached their apex after treatment for 4 h (Fig. 4).

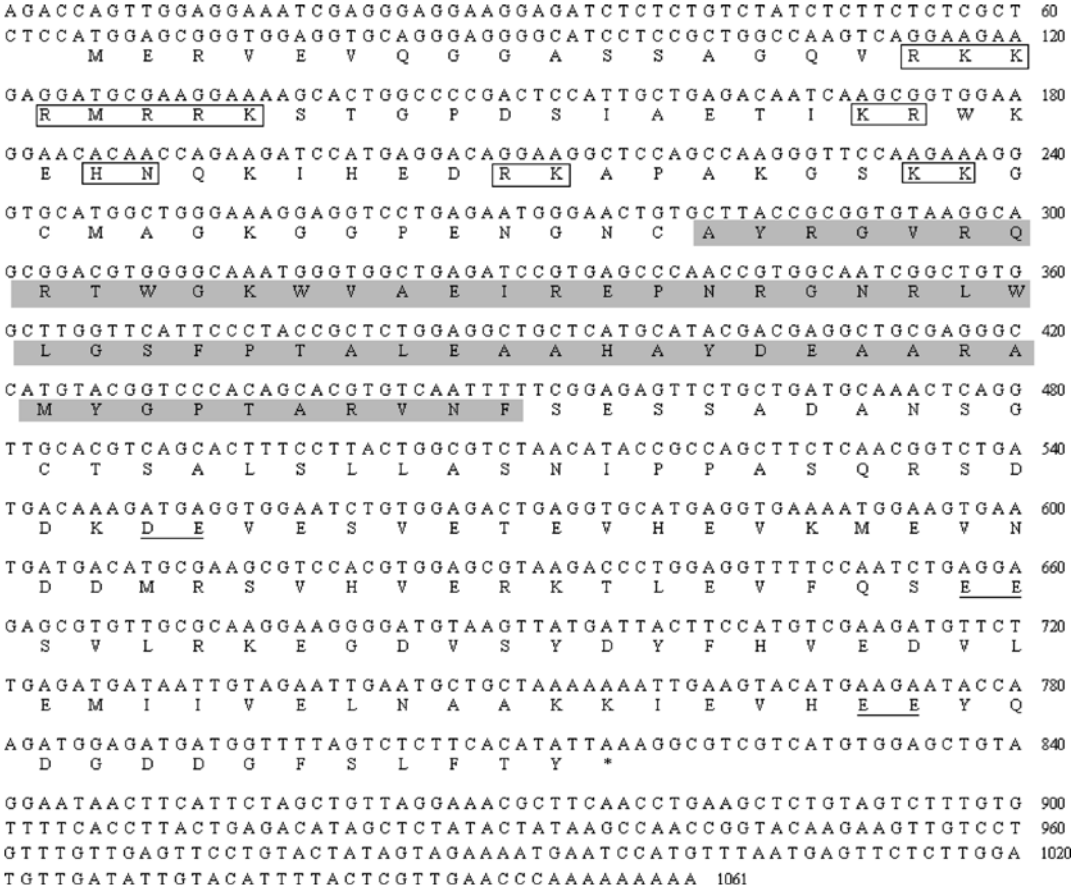
The above results suggested that *BeDREB1* and *BeDREB2* genes in Bermuda may be closely related to signaling pathways in response to environmental stresses.

3.3 Transcription activation of *BeDREBs*

The wild-type yeast strains after transformation with YepGAP-*BeDREB1* and YepGAP-*BeDREB2* plasmids grew well in 40 mmol/L 3-AT SD ($His^-/Trp^-/Ura^-$ deficient) culture medium, demonstrating that *BeDREB1* and *BeDREB2* ave transcription activation activity and can bind to the DREs activating the expression of the downstream reporter gene *HIS3* (Fig. 5). In contrast, mutated yeast cells could not grow normally in the same conditions, demonstrating that *BeDREB1* and *BeDREB2* cannot activate the mutant DRE (Fig. 5). All the results showed that *BeDREB1* and *BeDREB2* possess the properties of the DREB transcription activator family and are able to specifically bind to DRE, activating downstream gene expression.

4 Discussion

When plants are subjected to low-temperature, high-salt and drought conditions, the transcription of many genes, such as *rd29A*, *kin1*, *cor616* and *cor15A*, will increase greatly. All the members of DREB family have a remarkably mutual feature, i.e. all contain a conserved AP2/EREBP domain regulating the expression of those genes containing DRE or a core sequence (CCGAC) in their promoter region



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  R  M  R  R  K  S  T  G  P  D  S  I  A  E  T  I  K  R  W  K

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 R  T  W  G  K  W  V  A  E  I  R  E  P  N  R  G  N  R  L  W

GCTTGGTTCATTCCCTACCGCTCTGGAGGCTGCTCATGCATACGACGAGGCTGCGAGGGC 420
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The AP2/EREBP domain is shaded black. The N-terminal region, which might function as a nuclear-localization signal, is shown as open or closed boxes. There is an acidic C-terminal region, which might act as a transcriptional activation domain, is shown underlined. The nucleotide sequence had been submitted to the NCBI GenBank (GenBank accession No: AY462118)

Fig. 2 Nucleotide and deduced amino acid sequence of the cDNA encoding BeDREB2 from Bermuda grass under high-salt stress conditions

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BeDREB1  NCAYRGVRQRTUGKUVAEIREPNRGNRLWLGSFPTALEAAHAYDEAARANYGPTARVNFSESSA
BeDREB2  NCAYRGVRQRTUGKWVAEIREPNRGRRLWLGSFPTALEAAHAYDEAARANYGPTARVNFSESSA
DREB1A   HPIYRGVRRRNSGKWVCEVREPNKKTRLWLGTFQTAEHAARAHDVAALALRGRSACLNFADSAW
DREB1B   HPIYRGVRQRNSGKWVSEVREPNKKTRIWLGTFQTAEHAARAHDVAALALRGRSACLNFADSAW
DREB1C   HPIYRGVRQRNSGKWVSELREPNKKTRIWLGTFQTAEHAARAHDVAAIALRGRSACLNFADSAW
DREB2A   RCSFRGVRQRIWGKWVAELREPNRGSRLWLGTFPTAQEAASAYDEAAKANYGPLARLNFPRSDA
DREB2B   HCSFRGVRQRIWGKWVAEIREPKIGTRLWLGTFPTAEKAASAYDEAATANYGPLARLNFPQSVG
TaDREB   NCAVRGVRQRIWGKWVAEIREPNRGNRLWLGSFPTAVEAARAYDDAARANYGAKARVNFSEQSP
OsDREB   NCAYRGVRQRIWGKWVAEIREPNRGNRLWLGSFPTALEAAHAYDDAARANYGPTARVNFADNST
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The black background represents perfectly conserved amino acid resides. The partially conserved residues are shaded in different colors

Fig. 3 AP2/EREBP domain alignments of BeDREB1 (AY462117) and BeDREB2(AY462118) with those of other DREB proteins, including DREB1A(AB007787), DREB1B(AB007788), DREB1C(AB007789), DREB2A(AB007790) and DREB2B(AB007791) from *Arabidopsis thaliana*, TaDREB(AF303376) from *Triticum aestivum* and OsDREB(AY064403) from *Oryza sativa*

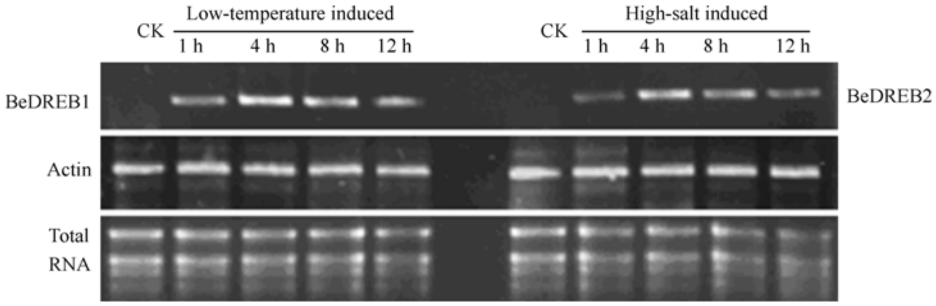
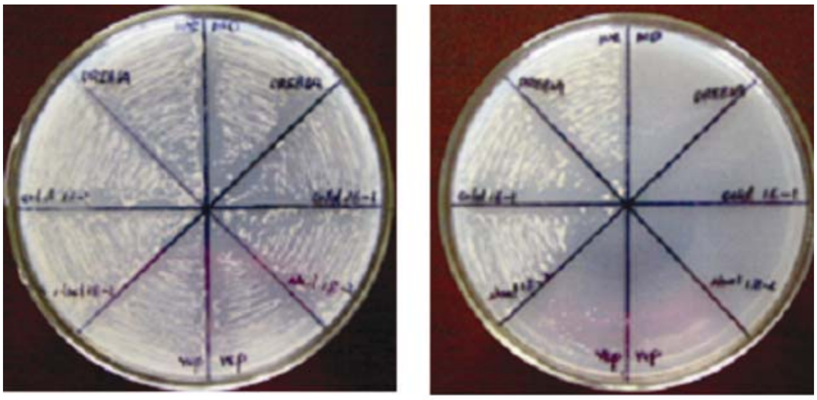
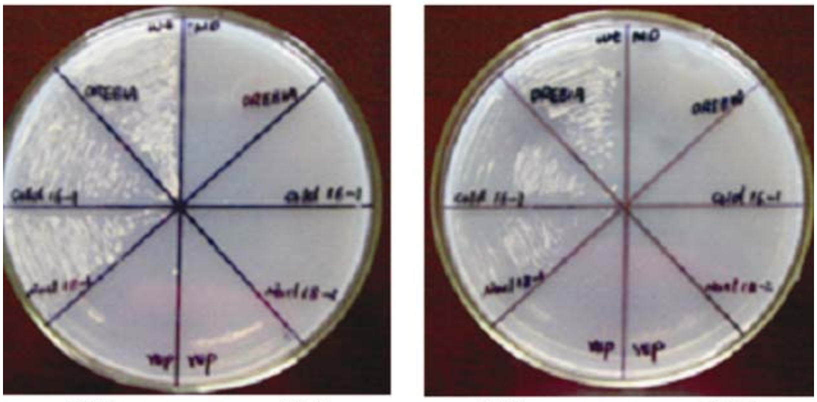


Fig. 4 Real-time PCR analysis of the expression patterns of BeDREB1 in low-temperature stressed Bermuda grass and BeDREB2 in high-salt stressed Bermuda grass in different inducible time



wtDRE mdDRE wtDRE mdDRE

0 mmol/L 3-AT 20 mmol/L 3-AT



wtDRE mdDRE wtDRE mdDRE

30 mmol/L 3-AT 40 mmol/L 3-AT

Fig. 5 Activation of *His* gene in yeast reporter cells by *BeDREB*1 or *BeDREB*2

through binding to DRE (Liu, et al., 1998). The conserved AP2/EREBP domain contains two regions: 20-amino-acid residue long basic and hydrophilic regions (YRG element) at the N-terminal. Three-dimensional structure analysis showed that there are three anti-parallel β-sheets in this domain, which play key roles in recognizing and binding to a variety of *trans*-elements. In the second β-sheet, valine and glutamate in position 14 and 19 are essential for specific binding to DRE (Cao et al., 2001; Sakuma et al., 2002). DREB1(A—C) has a conserved sequence, C/SEV/LR, between the position 14 and 19, whereas DREB2(A—B) has AEIR. Both DREB1 (A—C) and DREB2 (A—B) can specifically bind to DRE/CRT elements or their core sequences (A/GCCGAC), but the similarity of binding sequences are different. DREB1A can bind to CCCGAC, TCCGAC and ATCGAC, but DREB2 cannot. This difference affects the regulation of their gene expression in response to stresses (Sakuma et al., 2002). Following AP2/EREBP domain in DREB2, there is a serine- and threonine-rich region, which is supposed to regulate protein activity by phosphorylation and dephosphorylation. This is the additional difference between the DREB1 and DREB2 transcription activators.

BeDREB1 and *BeDREB2* isolated from low-temperature and high-salt treated Bermuda grass through RT-PCR and RACE-PCR also have valine and glutamate at position 14 and 19 in the conserved AP2/EREBR domain, which is the typical property of DREB family. The amino acid sequence between these two amino acids is an AEIR motif, indicating that these two genes belong to the DREB2 family. Previous studies on Arabidopsis DREB2A show that DREB2A can be induced by drought and high-salt conditions, participating in the osmotic stress-signaling pathway, but DREB2A needs post-transcription modification for activation. The activated DREB2A regulates the expression of a series of water-stress inducing and DREs-containing genes of plants leading to an increase in drought resistance (Medina et al., 1999; Nakashima et al., 2000). However, the induced gene expression patterns of the DREB2 family from different plants are not exactly the same. There are several DREB2 transcription activators reported, *TaDREB1, OsDREB2* and *HvDRF1*. *TaDREB1* is induced by low-temperature, drought and high-salt conditions and by ABA (Shen et al., 2003), whereas *HvDRF1* is induced by drought and high-salt conditions and by ABA (Xue and Loveridge, 2004). BeDREB1 and BeDREB2 were induced by low-temperature and high-salt conditions. Results of the induced expression pattern indicate that the expression of *BeDREB1* is related to the low-temperature stress-signaling pathway and that of *BeDREB2* to the osmotic stress-signaling pathway. Additionally, *BeDREB1* and *BeDREB2,* and *TaDREB1* and *OsDREB2* share high homology with each other. Yeast one-hybrid transcription activation assay showed that *BeDREB1* and *BeDREB2* could bind to DRE to activate the expression of downstream reporter genes, demonstrating that these two genes have similar functions to those from other plants. BeDREB1 and BeDREB2 proteins can regulate the expression of DRE-containing and stress-inducing genes, thus enhancing plant resistance to stresses. They can directly transcribe and activate the expression of DRE-containing and stress-inducing genes, which is different from the DREB2A transcription activators of Arabidopsis.

Sakuma and his colleagues reported three new members of the DREB subfamily in 2002, *DREB1D*, *DREB1 E* and *DREB1 F* and six *DREB2* homologous genes, *DREB2 C*, *DREB2D*, *DREB2 E*, *DREB2F*, *DREB2G* and *DREB2H* (Sakuma et al., 2002). Among the DREB1 transcription activator family, unlike *DREB1A* from Arabidopsis, *DREB1D* (*CBF4*) and *DREB1F* can be induced by high-salt but not by low-temperature conditions, suggesting their relationship with the osmotic stress-signaling pathway (Gilmour et al., 1998; Haake et al., 2002). Haake et al. (2002) reported that the expression of *DREB1D* was also induced by drought and ABA. Over-expression of *DREB1D* enhanced the drought resistance of plants. Recently, Hong and Kim isolated a DREB-like gene from hot pepper, *Ca-DREBLP1* (Hong and Kim, 2005). This gene is induced by drought and high-salt conditions and by mechanical damage, but not by low-temperature conditions, suggesting a new type of gene in the *DREB* subfamily.

This study is on the isolated *BeDREB1* and *BeDREB2* genes from qualified warm-season lawn grass. After analyzing the conserved domain of the deduced amino acid sequence, *BeDREB1* and *BeDREB2* were classified into the *DREB2* family. Yeast one-hybrid transcription activation assay demonstrated their transcription activation to DRE-containing promoters, indicating that the two genes participated in the low-temperature- and high-salt-induced signaling pathways. This research has laid an important foundation for genetic modification in lawn grass in terms of enhancing stress-resistance.

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