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Research article

Molecular characterization of a cDNA encoding DRE-binding transcription factor from dehydration-treated fibrous roots of sweetpotato

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Abstract

A new dehydration responsive element-binding (DREB) protein gene encoding for an AP2/EREBP-type transcription factor was isolated by screening of the cDNA library for dehydration-treated fibrous roots of sweetpotato (*Ipomoea batatas*). Its cDNA (referred to as *swDREB1*) fragment of 1206 bp was sequenced from, which a 257 amino acid residue protein was deduced with a predicted molecular weight of 28.17 kDa. A search of the protein BLAST database revealed that this protein can be classified as a typical member of a DREB subfamily. RT-PCR and northern analyses revealed diverse expression patterns of the *swDREB1* gene in various tissues of intact sweetpotato plant, and in leaves and fibrous roots exposed to different stresses. The *swDREB1* gene was highly expressed in stems and tuberous roots. In fibrous roots, its mRNA accumulation profiles clearly showed strong expression under various abiotic stress conditions such as dehydration, chilling, salt, methyl viologen (MV), and cadmium (Cd) treatment, whereas it did not respond to abscisic acid (ABA) or copper (Cu) treatment. The above results indicate that *swDREB1* may be involved in the process of the plant response to diverse abiotic stresses through an ABA-independent pathway.

Keywords: Abscisic acid; Dehydration; DREB; Fibrous root; Sweetpotato

1. Introduction

Drought stress acts as a key environmental factor that represents one of the principle limitations affecting plant species distribution and crop productivity [2,3]. Exposure to drought stress triggers many common reactions in plants [31]. This leads to cellular dehydration, which causes osmotic changes and removal of water from the cytoplasm into the extracellular space, resulting in decrease of the cytosolic and vacuolar volumes. Another consequence of this is the production of reactive oxygen species (ROS), which, in turn, may negatively affect cellular structures and metabolism. Therefore, drought triggers a wide variety of plant responses, including the regulation of gene expression, the accumulation of metabolites such as plant growth hormone (i.e., abscisic acid (ABA)) or osmotic active compounds, and the synthesis of specific proteins such as molecular chaperone and antioxidant enzymes [1].

The study of dehydration-induced genes in *Arabidopsis* has also revealed ABA-independent or ABA-dependent signal

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transduction pathways [27]. In *Arabidopsis*, *rd29A*, *rd29B*, *cor78*, and *lti78* genes are differentially induced under conditions of dehydration, cold, salt, and exogenous ABA. Dehydration responsive element (DRE: TACCGACAT) functions in the initial rapid response of *rd29A* to dehydration, salt, and low temperature [32,33]. The DRE is an essential *cis*-acting element for the regulation of *rd29A* induction in the ABA-independent response to dehydration in *Arabidopsis*. DRE-related motifs have been reported in promoters of several genes that are regulated by osmotic and low temperature stresses; these genes include *kin1*, *cor6.6*/*kin2*, and *rd17*/*cor47* in *Arabidopsis* [11,34]. In a recent study, 16 genes containing DRE or DRE-related core motifs (CCGAC) were identified in the promoters of dehydration-inducible *Arabidopsis* genes. This group of genes is a target sequence of the DREB1 or DREB2 transcription factors [26].

AP2/EREBP DNA-binding proteins include the DREB or CBF proteins that bind to DRE or C-repeats (CRT), respectively. The *Arabidopsis* genome encodes 145 AP2/EREBP proteins [24]. Among the AP2/EREBP proteins, the DREB subfamily is reported to comprise 55 genes in *Arabidopsis* [24]. A major transcriptional regulatory system is represented by DRE/C-repeat promoter sequences in stress-activated genes and DREBs/CBF factors that control stress-related gene expression [21,29]. A detailed analysis of expression showed that these factors may be associated with various stress conditions. For example, expressions of *DREB1A*/*CBF3*, *DREB1B*/*CBF1*, and *DREB1C*/*CBF2* are induced by low temperature. *DREB1D*/*CBF4*, *DREB2A*, and *DREB2B* are induced by salt and dehydration. *DREB1F*, *DREB2C*, *DREB2D*, and *DREB2F* are slightly induced by high salt treatment, whereas *DREB2E* is slightly induced by ABA treatment. However, the expressions of *DREB1E*, *DREB2G*, and *DREB2H* have not been detected upon exposure to various stress conditions [7,21,22,24,28].

Sweetpotato (*Ipomoea batatas*) is known as a relatively drought-resistant crop, and it is one of the most important root crops in the world. However, sweetpotato has not been investigated in relation to the molecular basis of its drought stress tolerance, even though it is quite tolerant to unfavorable growth conditions, including drought conditions. Moreover, no reports have been published on DRE-binding transcription factor in sweetpotato. The roots of plants are the primary sensors of drought and salt stress [4,25]. Therefore, in this study we have cloned a new *DREB* gene from the fibrous roots of sweetpotato treated with dehydration. To analyze the roles of *swDREB1* in adaptation to stress in sweetpotato plants, its expression patterns were examined in different tissues under various stresses.

2. Results

2.1. Isolation and sequence analysis of the swDREB1

To isolate the cDNA clone encoding DREB, a cDNA library was constructed from dehydration-treated fibrous roots of sweetpotato and screened with a probe produced by PCR using a DREB-conserved region. A positive clone containing the largest insert was designated as an *swDREB1*, and was selected for further analysis.

Its cDNA is 1206 bp in length and encodes a deduced 257 amino acid residue protein with a predicted molecular mass of 28.17 kDa. Database searches revealed that the amino acid sequence of the SwDREB1 protein contains a conserved, 63 amino acid, DNA-binding domain that is present in a large family of plant DNA-binding proteins. Its N-terminal includes a basic residue, PKKRAGRKKFRETRHP, which might function as a nuclear localization signal (NLS). An acidic region in its C-terminal (AP2/EREBP domain) was thought to be an activation domain for the transcription of *DRE*/*CRT* genes (Fig. 1A). The swDREB1 protein also contains the DSAWRL and LWSF motifs (Figs. 1 and 2). Alignment and comparison of its amino acid sequence with those of other *DREB* genes isolated from various plants showed that it shares 67% similarity with *CaCBF1B* and *CaDREBLP1*, and 61% similarity with *AtDREB1D*/*CBF4* (Fig. 2A). Phylogenic tree analysis of plant DREB/CBF protein sequences indicated that the swDREB1 shares high homology with those in various plant species such as tomato (LeCBF1), hot pepper (CaCBF1B and CaDREBLP1), cotton (GhDREB1A) (Fig. 2B). Among *Arabidopsis* DREBs, swDREB1 is more closely related with AtDREB1/CBF than AtDREB2. Therefore, we suggest that *swDREB1* can be classified as a member of the DREB subfamily, and will likely play an important role in abiotic stress-responsive gene expression.

2.2. Genomic organization of swDREB1

To elucidate the genomic organization of *swDREB1*, the 3′-UTR region was amplified from cDNAs, cloned to pGEM-T Easy vector, sequenced, and used as probes. Southern blot analysis of genomic DNA digested with EcoRI, EcoRV, and HindIII is shown in Fig. 3. A single hybridizing band was observed, indicating that one copy of *swDREB1* may exist in the sweetpotato genome.

2.3. Differential expression of swDREB1 in intact sweetpotato tissues

The expression patterns of the *swDREB1* gene were investigated in various whole plant tissues (L, leaf; S, stem; TR, tuberous root; FR, fibrous root; TPR, thick pigmented root) by RT-PCR and northern blot analyses (Fig. 4). The RT-PCR results were well correlated with those of northern blot analysis proving the validity of RT-PCR analysis. The results demonstrate that there is considerable variation in the levels of *swDREB1* expression in different sweetpotato tissues. The *swDREB1* gene was strongly expressed in stem, tuberous root and fibrous root tissues, whereas it was weakly expressed in leaf and thick pigmented root tissues.

2.4. Expression patterns of the swDREB1 to various abiotic stresses in leaves and fibrous roots

In order to investigate the *swDREB1* gene expression under various abiotic stresses such as dehydration, ABA, cold, salt,