Plant Physiology and Biochemistry 60 (2012) 98–108

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Ectopic expression of dehydration responsive element binding proteins (StDREB2) confers higher tolerance to salt stress in potato

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ARTICLE INFO

Article history:
Received 17 March 2012
Accepted 26 July 2012
Available online 8 August 2012

Keywords:
Abiotic stresses
DREB
Solanum tuberosum
Transcription factor
Transgenic plants

ABSTRACT

Dehydration responsive element binding proteins (DREB) are members of a larger family of transcription factors, many of which have been reported to contribute to plant responses to abiotic stresses in several species. While, little is known about their role in potato (*Solanum tuberosum*). This report describes the cloning and characterization of a DREB transcription factor cDNA, StDREB2, isolated from potato (cv Nicola) plants submitted to salt treatment. Based on a multiple sequence alignment, this protein was classified into the A-5 group of DREB subfamily. Expression studies revealed that *StDREB2* was induced in leaves, roots and stems upon various abiotic stresses and in response to exogenous treatment with abscisic acid (ABA). In agreement with this expression pattern, over-expression of *StDREB2* in transgenic potato plants resulted in enhanced tolerance to salt stress. These data suggest that the isolated *StDREB2* encodes a functional protein involved in plant response to different abiotic stresses.

An electrophoretic mobility shift assay (EMSA) indicated that the StDREB2 protein bound specifically to the DRE core element (ACCGAGA) in vitro. Moreover, Semi quantitative RT-PCR analysis revealed that the transcript level of a putative target gene i.e. δ^1-pyrroline-5-carboxylate synthase (P5CS) was up-regulated in transgenic plants submitted to salt stress conditions. A concomitant increase in proline accumulation was also observed under these conditions.

Taking together, all these data suggest that *StDREB2* takes part in the processes underlying plant responses to abiotic stresses probably via the regulation of ABA hormone signaling and through a mechanism allowing proline synthesis.

1. Introduction

Environmental stresses, such as drought, high salt and low temperature adversely affect plants growth and their productivity. Response to abiotic stresses is a very complex phenomenon as various stages of plant development can be affected by a particular stress and often several stresses simultaneously affects the plant [1,2]. To better understand how plants adapt to various stresses, it is important to explore how different response pathways interact with each other.

Many transcriptional factors involved in these stress-resistance pathways have been identified, among which the drought-responsive element binding factors (*DREBs*) and C-repeat Binding Factors (*CBF*) that belong to AP2/ERF super family [3]. The *DREB*/*CBFs* transcription factors are involved in plant response to environmental stresses such as cold and drought [3,4]. They are considered as the best studied group of transcriptional factors involved in abiotic stress response. The DREB factors activate the expression of several target genes that are responsible for controlling correlated characters such as osmoprotection and metabolism [2,5]. These proteins can specifically bind to Dehydration-responsive element (DRE)/C-repeat (CRT), and mediate transcription of target genes [3]. The DRE/CRT is one of the major cis-acting elements which function in either ABA-responsive or non-responsive gene expression during abiotic stresses [2,6].

DREB/*CBF*-like gene was firstly isolated in *Arabidopsis* [3] and subsequently identified in a wide variety of plants, including wheat

Abbreviations: DREB, Dehydration Responsive Element Binding proteins; CBF, C-repeat Binding Factor; ABA, Abscisic Acid; ERF, Ethylene Response Factors; P5CS, δ-pyrroline-5-Carboxylate Synthase.

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http://dx.doi.org/10.1016/j.plaphy.2012.07.029

(*Triticum aestivum* L.) [7], barley (*Hordeum vulgare* L.) [8] and rice (*Oryza sativa* L.) [9]. The CBF/DREB group, previously classified in the A subfamily [10] of the ERF superfamily, was then divided into different groups I, II, III (DREB1) and IV (DREB2), based on the sequence similarities of the AP2/ERF domain and conservation of other specific motifs present in the ERF proteins [11]. DREB factors showed variation in some conserved motifs and in their biological functions in different species. They are also involved in separate signal transduction pathways involved in abiotic stress responses [2,9].

The differential transcript regulation and functional analysis of *DREB* genes was reviewed in Agarwal et al. [12].

Expression of DREB1-type genes was specifically induced by low-temperature stress in *Arabidopsis* and rice [9,10]. In contrast, DREB2-type genes responded to dehydration and high-salt stresses [9]. Recently, a DREB1-type gene from hot pepper (*Ca-DREBLP1*), was found to be induced by water-deficit and salt stress and to a lesser extent by mechanical wounding whereas it was not induced by cold stress [12,13]. Other DREB1-type genes, such as soybean (*GmDREB2*) and *Caragana korshinskii* (*CkDREB*), were induced by drought, high-salt and low-temperature stresses, as well as by ABA treatment [14,15]. Wheat *TaDREB1* and *WDREB2*, maize *ZmDREB2A*, and pearl millet PgDREB2, DREB2-type genes are responsive to cold stress as well, whereas foxtail millet SiDREB2 was not [2,9,16–20]. The maize DREB2-type gene (*ZmDREB2A*), was shown also to accumulate under heat stress at seedling stage [19].

It was shown that DREB factors activate the stress response through DREs in ABA-independent manner [21]. However, DREB proteins have been also reported to be engaged in an ABA-mediated gene expression pathway [22,23]. Recently, DREB1A/CBF3, DREB2A, and DREB2C proteins have been reported to physically interact with AREB/ABF proteins.

[2,23,24], which supports the view that DREB/CBFs and AREB/ABFs may interact to control ABA-regulated gene expression. Moreover, in soybean, the expression pattern of the *GmDREB2* gene suggests that it acts as an overlap point and might take part in both ABA-dependent and independent pathways, simultaneously [2,14,21]. Similarly, the expression of *WDREB2* from wheat was shown to be responsive to exogenous ABA treatment [17,21,23], whereas transgenic tobacco plants overexpressing this gene were hypersensitive to exogenous ABA during post germination growth compared with wild-type tobacco [21,25], thereby suggesting that wheat DREB2 might contribute indirectly to the development of abiotic stress tolerance through an increase in ABA sensitivity. In the case of maize (*Zea mays*), DRE-binding AP2/ERF domain factors, DBF1 and DBF2, are shown to be not only ABA inducible but they also regulate ABA response in vivo [23,26]. All, these observations suggest that DRE/CRT-regulated expression of some genes may be ABA dependent [2]. These studies highlight that there is a crosstalk during stress signaling, mediated by a synergistic effect of ABA and drought/salt stress, for the regulation of stress responsive genes [27].

In addition, *DREB*s are considered as candidate genes for stress tolerance engineering and also in marker-assisted selection (MAS) to develop stress tolerant crop varieties [2].

Many DREB1-type genes inserted into plants by transformation were capable of improving multiple abiotic stress tolerances in agricultural crops including tobacco [28], wheat [29], rice [9], *Chrysanthemum* [30], *C. korshinskii* [15], and potato [31].

Potato (*Solanum tuberosum* L.) is a major food crop worldwide with premier economic importance. However, due to its sparse and shallow root system, this species is very sensitive to environmental stresses such as high salinity, drought, and severe temperature changes. Consequently, tuber yield can be considerably reduced by such stresses, and efforts to investigate the mechanisms of molecular adaptation to stresses aiming to open new leads toward strengthening stress tolerance are of fundamental importance for potato production. Up to date, no *DREB*s from *S. tuberosum* have been characterized. However, increased salt tolerance has been observed in transgenic potato lines harboring *Arabidopsis rd29A*::*DREB1A* gene [32,33]. Moreover, the *Arabidopsis rd29A*::*DREB1A* seemed also able to improve the response of transgenic potato plants to freezing [34], suggesting that the control of tolerance to abiotic stress in potato, is mediated by DREB factors. Similarly, ectopic *AtCBF1* overexpression enhanced freezing tolerance and induced cold acclimation-associated physiological modifications in potato [31].

The present report describes the isolation and functional characterization of a DREB gene (*StDREB2*) in potato. It is shown that *StDREB2* transcript accumulation rapidly increases in response to salt, cold and drought treatment suggesting an active role for this gene in the adaptation mechanisms to abiotic stresses. The data also indicate that *StDREB2* expression is dependent on an active abscisic acid (ABA) signaling pathway. In line with the abiotic stress-associated pattern of expression, transgenic potato plants over-expressing the *StDREB2* gene, exhibited enhanced tolerance to salt stress.

2. Results

2.1. Isolation and sequence analysis of the StDREB2 full length cDNA

A full-length cDNA clone corresponding to a potato *DREB* gene was obtained using the Gateway technology which is a universal cloning method based on the site-specific recombination properties of lambda bacteriophage [35]. To enable recombinational cloning and efficient selection of entry or expression clones, most Gateway® vectors contain two att sites (from lambda bacteriophage) flanking the cloning cassette. After a recombination reaction, this cassette is replaced by the gene of interest to generate the entry clone or the expression clone.

The isolated cDNA, designated *StDREB2* (*S. tuberosum* drought-responsive element binding factor 2; GenBank ID: JN125858) possesses an open reading frame (ORF) of 438 bp encoding a putative protein of 146 amino acids. A sequence alignment revealed the presence of the conserved AP2/ERF central 58 amino acids located in the N-terminal position and harboring valine residue at position 14 and glutamic acid at position 19 which both characterize DREB members [10] (Fig. 1A). Transcription factors, that share functionally important domains involved in transcriptional activity and protein–protein interactions can be classified in the same subgroup and the presence of common motifs within a subgroup may suggest shared functions [36]. *In silico* analysis of *StDREB2* encoded protein indicated that it shares high similarities with several DREB proteins belonging to class II [11] that correspond to A5 group [10]. Indeed, the sequence comparison of the overall amino acid sequence shows that StDREB2 share 66, 70, 72 and 57% amino acid similarity with *Glycine max* L. GmDREB1 and GmDREB2, the *Gossypium hirsutum* GhDBP1, and *Arabidopsis* RAP2.1 respectively (Fig. 1A).

Moreover, StDREB2 contains the EAR repressor motif located in the C-terminal domain (ERF-associated amphiphilic repression) defined as CMII-2 [11].

A phylogenetic analysis (Fig. 1B) performed using the MEGA4.0 program allowed classification of *StDREB2* into the A-5 subgroup of DREB subfamily [10], besides GhDBP1 (*G. hirsutum*), RAP2.1 (*Arabidopsis*), GmDREB1 and GmDREB2 (*G. max* L).

2.2. Expression pattern of the StDREB2 gene in plants submitted to salt stress

To determine the tissue-specific expression pattern of the *StDREB2* gene under different stress conditions, we monitored the