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Isolation and characterization of StERF transcription factor genes from potato (*Solanum tuberosum* L.)

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Abbreviations :
ABA, Abscisic acid
ERF, Ethylene response factor
MeJA, Methyl jasmonate
PR, Pathogenesis-related
qRT-PCR, Quantitative real-time polymerase chain reaction
SA, Salicylic acid

ABSTRACT

Ethylene response factor (ERF) is a major subfamily of the AP2/ERF family and plays significant roles in the regulation of abiotic- and biotic-stress responses. ERF proteins can interact with the GCC-box cis-element and then initiate a transcriptional cascade activating downstream ethylene response and enhancing plant stress tolerance. In this research, we cloned five *StERF* genes from potato (*Solanum tuberosum* L.). The expressional analysis of *StERF* genes revealed that they showed tissue- or organ-specific expression patterns and the expression levels in leaf, stem, root, flower, and tuber were different. The assays of quantitative real-time polymerase chain reaction (qRT-PCR) and the reverse transcription-PCR (RT-PCR) showed that the expression of five *StERF* genes was regulated by ethephon, methyl jasmonate (MeJA), salt and drought stress. The result from the yeast one-hybrid experiment showed that five StERFs had trans-activation activity and could specifically bind to the GCC-box cis-elements. The StERFs responded to abiotic factors and hormones suggested that they possibly had diverse roles in stress and hormone regulation of potato.

1. Introduction

Plants, including potato, respond to environmental stresses with an array of biochemical and physiological adaptations, which involve complex multiple signaling pathways, genes and gene products. Regulating the expression of stress-related genes is one of the most effective regulatory pathways for plants to adapt to adverse environments [1]. A mass of genes has been reported that allow plants to tolerate and overcome unfavorable circumstances [2–5].

In plant genomes, approximately 7% of the coding sequences are assigned to transcription factors (TFs) [6], and many of them are immediate-early and abiotic stress-responsive genes [7], which have been known to play crucial roles in response to important abiotic stress factors, including drought, high salinity, high osmolarity, extreme temperature, and phytohormone [8,9].

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Sakuma et al. [10] classified AP2/ERF transcription factors into five subfamilies: AP2 (APETALA2), RAV (related to ABI3/VP1), DREB (dehydration-responsive-element binding protein), ERF (ethylene-responsive factor), and others, according to the number and similarity of the DNA-binding domains (DBD). ERFs play significant roles in the regulation of abiotic- and biotic-stress responses. ERE (ethylene-responsive element) binding factor (ERF) proteins (formerly known as ERE binding proteins [EREBPs]) were isolated as GCC box binding proteins from tobacco (*Nicotiana tabacum*) [11], and their expression pattern was investigated in detail [12]. ERE binding proteins contain a highly conserved DNA binding domain (designated as the ERF domain) [13] consisting of 58 or 59 amino acids [11]. The AP2 domain (ERF domain) was centered on the base amino acid sequence AAEIRD**RR*R*WLGT*DTAEEAA where the underlined WLG amino acids were diametrically required for AP2/ERF domain binding to DNA and the * represented non-specifically conserved amino acids [10,14]. Pti4 was an ERF transcription factor that was first isolated by its interaction with the kinase Pto, which conferred resistance to *Pseudomonas syringae* pv. tomato expressing the avirulence gene *AvrPto* [15].

GCC box widely exists in a large number of gene promoters. ERF can interact with the GCC box in the promoter of genes and activate downstream gene expression. The analysis of expression profile in the transgenic plants with *ERF104* gene overexpression evidenced that the expression level of 534 genes raised more than 3-fold changes with the strongest induction (~1000 fold) for two *PDF1.2* genes, and the 1-kb upstream regions of genes up-regulated >10-fold were enriched in GCC elements [16]. Moreover, the ERF proteins can regulate the biosynthesis of metabolites, such as wax [17], ethylene [18], jasmonate [19], nicotine [20], and gibberellin [21,22]. The result from overexpressed *AtERF98* in a Col-0 background plants in *Arabidopsis* indicated that AtERF98 played an important role in regulating the biosynthesis of ascorbic acid [23].

At present, some ERF genes in plants have been profiled; however, their function was not well explored. In this study, five *StERF* genes (*ERF1-4* and *Pti4*) in potato were completely identified and characterized, which suggested that StERFs played important roles in potato.

2. Materials and methods

2.1. Plant materials and growth conditions

Potato (*Solanum tuberosum* L.) cultivar 'Zihuabai' was used in the experiment. Plants were grown in *in vitro* culture in a glass bottle (7 cm diameter and 9 cm height) at (21 ± 2) °C under a 16/8 h light/dark photoperiod on 50 ml MS media containing 3% (w/v) sucrose

2.2. Phylogenetic analysis

Full-length sequences of five *StERF* genes were originally retrieved from NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank), and the GenBank accession No. as follows: *StERF*1 (JN125860), *StERF*2 (JN711505), *StERF*3 (JN125857), *StERF*4 (JN125859) and *Pti4* (EU851735). Protein sequences were obtained from potato genome resource (http://potato.plantbiology.msu.edu/index.shtml). *Arabidopsis ERF* genes examined herein were designated *AtERF1-5* and were previously noted by Nakano et al. [14]. The phylogenetic tree was generated by ClustalX2 using default parameters of the neighbor-joining method in MEGA (version 5.0). The StERFs subcellular localization was analyzed using PSORT (http://www.psort.org/). The StERFs signal peptide (SP) was analyzed using Signal P4.1 Serve (http://www.cbs.dtu.dk/services/SignalP/). The domain of the transcription factor was analyzed using the Pfam online data (http://pfam.xfam.org/family/).

2.3. Hormone and stress treatments

For plant hormone treatment, four-leaf-stage plants were grown in solid MS media, then the plants were sprayed with 100 μM MeJA and 100 μM ethephon (5 ml), respectively, and the control was sprayed with distilled water. For abiotic stress, four-leaf-stage plants were grown in liquid MS media as described above, then the liquid media were removed followed by adding 50 ml of liquid MS media containing 200 mM NaCl and 20% PEG6000, respectively, and by adding distilled water as the control. Plant leaves were collected after treatment (1 h, 2 h, 6 h and 12 h) and immediately frozen in liquid nitrogen, then stored at −80 °C. Every experiment was repeated three times.

2.4. RNA isolation, cDNA synthesis, and gene expression analysis

Sprouted seed tubers (about 100 g weight) of potato cultivar 'Zihuabai' were planted in pots with three replicates in greenhouse under natural light conditions in Gansu Agricultural University. Leaves, stems, flowers and roots were harvested from 38-day potato plants, and tuber from 60-day potato plants, and then stored at −80 °C until use. Total RNA was isolated using Trizol Reagent (Invitrogen, #15596026) according to the manufacturer's instructions and digested with DNase I (TaKaRa, #2270A). Five micrograms of total RNA were used for cDNA first strand synthesis according to the manufacturer's instructions of One Step PrimeScript® cDNA Synthesis Kit (TaKaRa). The default cycling conditions (3 min at 94 °C and 35 cycles of 94 °C for 30 s, 55 °C for 1 min, 72 °C for 1 min and 10 min final extension at 72 °C) were performed using T100™ Thermal Cycler, BIO-RAD PCR System. The PCR products were electrophoresed on a 1.0% agarose gel. The *StERF*-specific primers used in RT-PCR were listed in Table 1. All samples were compared with the endogenous reference gene *ef1a*.

For expression analysis of *StERF* genes under stress treatments, qRT-PCR was performed in 20-μl reaction mixtures with the SYBR® Premix Ex Taq™ II Kit (TaKaRa, #DRR047A) and 10 μM of each primer. The *ef1a* was as an internal control gene. The gene-specific primers were listed in Table 1. Reactions were conducted on the ABI3000 System (Applied Biosystems 3000 Real-Time PCR) using