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Characterization of two cDNAs (ERD11 and ERD13) for dehydration-inducible genes that encode putative glutathione *S*-transferases in *Arabidopsis thaliana* L.

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Two cDNA clones, designated ERD11 and ERD13, isolated from a cDNA library from *Arabidopsis thaliana* L. plants dehydrated for 1 h were sequenced and characterized. These clones encoded polypeptides that were homologous to glutathione *S*-transferases of tobacco and maize. Genomic Southern hybridization suggested that there are a few additional genes showing high similarity to the ERD11 gene in the *Arabidopsis* genome. The expression of the genes for ERD11 and ERD13 was induced by dehydration, but was not affected by the application of four plant growth regulators, 2,4-dichlorophenoxyacetic acid 6-benzylaminopurine, abscisic acid, or gibberellic acid.

Arabidopsis thaliana L.; Dehydration-inducible gene; Glutathione *S*-transferase (GST)

1. INTRODUCTION

Glutathione *S*-transferases (GSTs, EC 2.5.1.18) are enzymes that catalyze the conjugation of glutathione with a large number of hydrophobic, electrophilic compounds. GSTs have been identified in many eukaryotes, including plants [1,2]. The best-known function of GSTs is the detoxification of xenobiotic compounds. In insects, GSTs are reported to have an important role in the acquired resistance to insecticides [3,4]. In mammals, the cytochrome P-450 system and GSTs are reported to play pivotal roles in determining the formation or persistence of cytotoxic or carcinogenic intermediates that arise during the metabolism of xenobiotics [5,6]; and in plants, maize GSTs (GST I and II) are reported to detoxify some herbicides [7]. While several other functions of GSTs have been postulated [8–10], the precise physiological roles remain unknown.

We investigated the early responses of plants to dehydration at the molecular level, and isolated 27 cDNA clones whose gene-expression was induced by dehydration for 1 h in *Arabidopsis thaliana*. These clones were classified into 16 groups (ERD clones: cDNA clones gene-expression for which are *e*arly *r*esponsive to *d*ehydration-stress) based on Southern blot hybridization [11]. We report on two of these clones, ERD11 and ERD13, which may correspond to cDNAs for glutathione *S*-transferases, and we discuss the possible function of these dehydration-inducible GSTs.

2. MATERIALS AND METHODS

2.1. *Plant materials*

One set of seeds of *A. thaliana* L. (Columbia ecotype) were sown on vermiculite beds and grown at 22°C for 4–5 weeks under continuous illumination of 2,500 lux. Another set of seeds of *A. thaliana* were surface-sterilized with a solution of sodium hypochlorite (1% w/v, WAKO, Osaka, Japan) for 15 min, rinsed 3 times with sterilized distilled water, sown on GM agar (0.8% w/v, Bacto-agar, Difco, Detroit, MI) medium [12], and were then grown for 4–5 weeks.

2.2. *Isolation and hybridization of DNA and RNA*

RNA and genomic DNA were isolated from whole plants of *A. thaliana*, which were harvested prior to bolting, as described earlier [13]. cDNA fragments of ERD11 and ERD13 were labelled by random priming with [^{32}P]dCTP (110 TBq/mmol, Amersham, Aylesbury, UK) using a kit from Boehringer Mannheim (Mannheim, Germany) and were used for hybridization. Hybridization of DNA and RNA was performed as described earlier [13].

2.3. *cDNA sequencing and sequence analysis*

The sequencing of the cDNAs was determined using a DNA sequencer (Model 373A, ABI, San Jose, CA). Nucleotide sequences and deduced amino acid sequences were analyzed using the GENETYX software system (Software Development Co., Tokyo, Japan).

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The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank Nucleotide Sequence Databases with the following accession numbers D17672 and D17673.

2.4. *Dehydration and treatment with plant growth regulators*

Whole plants of *A. thaliana* grown on vermiculite beds were harvested, washed gently, and subjected to dehydration on chromatography paper (3MM, Whatman, Maidstone, England). Whole plants of *A. thaliana* axenically grown on GM agar medium were transferred to Petri dishes (diameter 9 cm, Falcon, Lincoln Park, NJ) that contained 20 ml of liquid GM medium with either 10^{-4} M 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BA), abscisic acid (ABA), or gibberellic acid (GA_3), and were then incubated for 10 h.

3. RESULTS AND DISCUSSION

3.1. *Sequence analysis of ERD11 and ERD13*

Fig. 1A and B show the nucleotide sequences and the corresponding amino acid sequences of cDNA clones for ERD11 and ERD13, respectively. ERD11 cDNA consists of 883-bp nucleotides encoding a polypeptide of 208 amino acids having a predicted molecular weight of 23,546. ERD13 cDNA consists of 862-bp nucleotides encoding a polypeptide of 215 amino acids having a predicted molecular weight of 24,230. ERD11 cDNA contains the polyadenylation consensus sequence, AATAAA, while ERD13 cDNA contains AATAAG.

The deduced amino acid sequences of ERD11 and ERD13 were compared to those compiled in databases and were found to exhibit significant homologies to those of GSTs in a variety of eukaryotes. ERD11 protein is most closely related (56.5%) to the tobacco parB protein from the database sequence, which is the product of an auxin-inducible gene [14]. The recombinant parB protein expressed in *E. coli* was shown to possess GST activity [14]. ERD13 protein is most closely related (44.1%) to the maize GTS III sequence [15]. ERD11 and ERD13 are 39.6% identical at the amino acid level. The primary sequences of these four GSTs are aligned in Fig. 1C. Common amino acids (indicated by asterisks in Fig. 1C) are distributed throughout these four sequences. The well-conserved region of amino acids 48–76 corresponds to the possible functional domain for the enzymatic activity of GSTs suggested by Takahashi and Nagata [14].

3.2. *Identification of genes for ERD11 and ERD13*

Genomic Southern blot hybridization was carried out to identify GST genes in the *Arabidopsis* genome (Fig. 2). ERD11 cDNA has one internal *Hin*dIII restriction site, two *Eco*RI sites, and no internal restriction site for *Pst*I, *Xba*I, and *Bam*HI restriction enzymes. We observed a few faint bands in addition to the intense bands in every lane (Fig. 2A), indicating that there are a few genes that are highly similar to the ERD11 gene. ERD13 cDNA has one restriction site for *Hin*dIII and none for *Pst*I, *Xba*I, *Bam*HI, and *Eco*RI enzymes. Since only distinct bands were observed (Fig. 2B), there appeared to be no genes that are highly similar to the ERD13 gene.

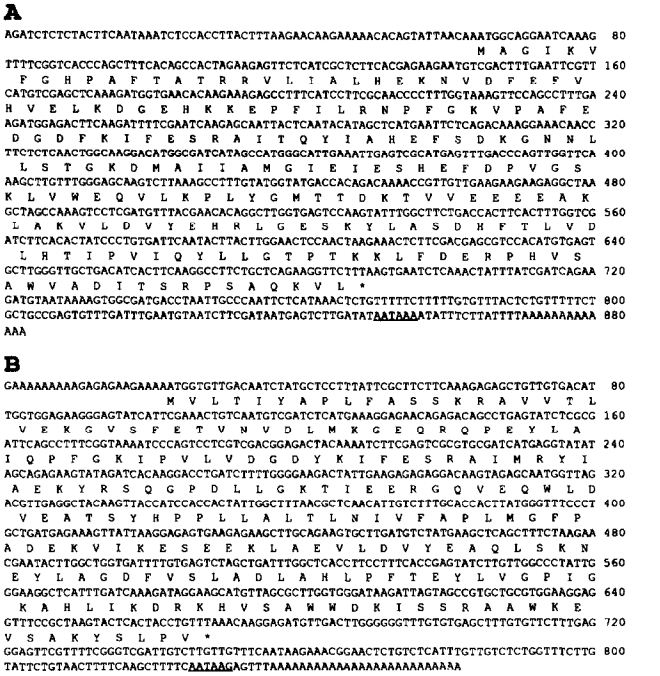

Fig. 1. Nucleotide and deduced amino acid sequences of cDNAs for ERD11 (A) and ERD13 (B). The nucleotide sequences contain the coding regions and the 5′- and 3′-non-coding regions. The amino acid sequences of the putative coding regions are shown beneath the nucleotide sequences. The conserved polyadenylation sequence (AATAAA) and the similar sequence (AATAAG) are underlined. (C) Comparison of the deduced amino acid sequences of ERD11 and ERD13, the maize GST III [15], and the tobacco parB [14]. Dots represent amino acid residues identical to those for ERD11 and dashes indicate gaps introduced to maximize alignment. Asterisks represent amino acid residues common to the four sequences.

3.3. *Expression of the genes for ERD11 and ERD13*

Northern blot analysis was used to estimate the expression of genes for ERD11 and ERD13. Gene-expression of both ERD11 and ERD13 was induced by dehydration of plants for 1 h, and the maximum levels of accumulation of mRNAs were observed after 10 h of dehydration (Fig. 3A). We tested the effects of four

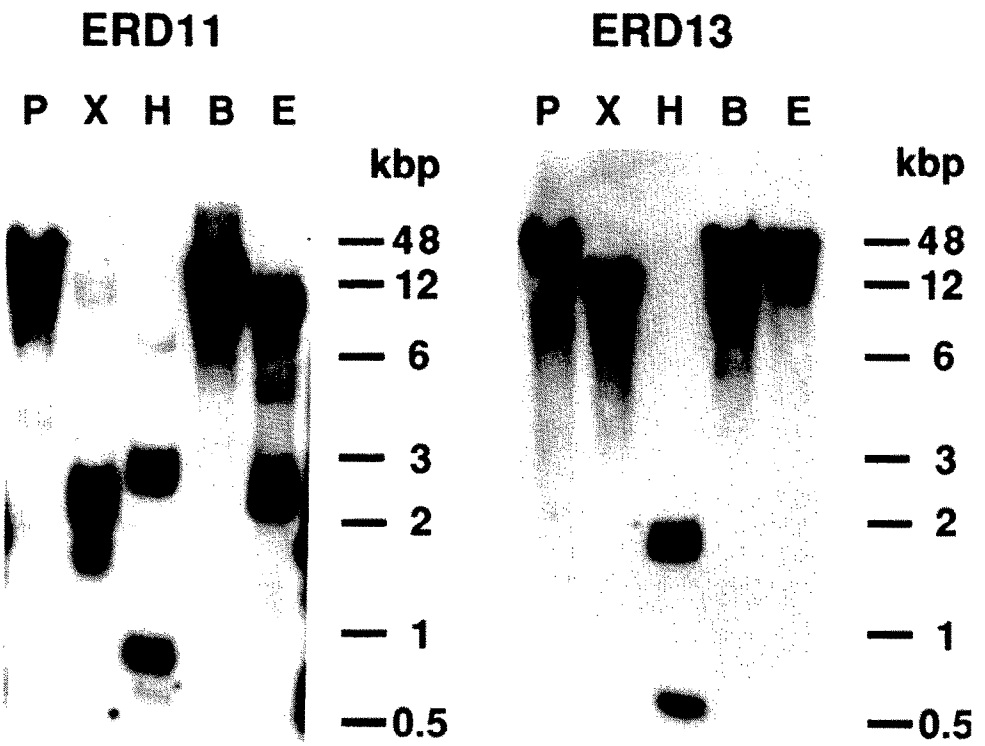

Fig. 2. Southern blot analysis of ERD11 and ERD13 genomic sequences. Genomic DNA was digested with *Pst*I (P), *Xba*I (X), *Hin*dIII (H), *Bam*HI (B), and *Eco*RI (E); and was then fractionated through 0.7% agarose gels and transferred to nitrocellulose membranes. Filters were hybridized with ^{32}P-labelled cDNA fragments of ERD11 (left) and ERD13 (right). The sizes of DNA markers are indicated in kbp.

plant growth regulators on the expression of genes for ERD11 and ERD13, since the expression of parB gene was reported to be inducible by auxin [14]. In the case of ERD11, no induction was observed with any of the plant growth regulators tested (Fig. 3B). In contrast, the expression of the gene for ERD13 was induced when axenic whole plants, grown on agar medium, were incubated to liquid medium, without regard to the absence or presence of plant growth regulators (Fig. 3B). While the reason for this induction of gene-expression for ERD13 is unknown, the ERD13-gene-expression appeared to be more sensitive to the change of medium than the ERD11-gene. No auxin-specific induction was observed in the expression of either ERD gene. The finding that these genes are inducible by dehydration and non-inducible by auxin indicates that their expression is unique among the GST genes reported. The expression of GST genes has been observed in senescent flower petals of carnations [16], and has been shown to be induced by ethylene in both caranation and *Arabidopsis* [17,18]. Both the developmental expression and the effect of ethylene on the expression of the genes for ERD11 and ERD13 remain to be determined.

The functions of ERD11 and ERD13 products in dehydrated plants remain unclear. One possible role of these proteins is the detoxification of toxic compounds formed during dehydration-stress. We isolated a cDNA clone for soluble epoxide hydrolase in *A. thaliana* whose gene-expression was slightly induced by dehydration-stress (manuscript in preparation). In mammals, epoxide hydrolases are known to be involved in detoxification of xenobiotics in addition to the cytochrome P-450 system and GSTs [5]. Future investigations should use antisense techniques to better understand these functions.

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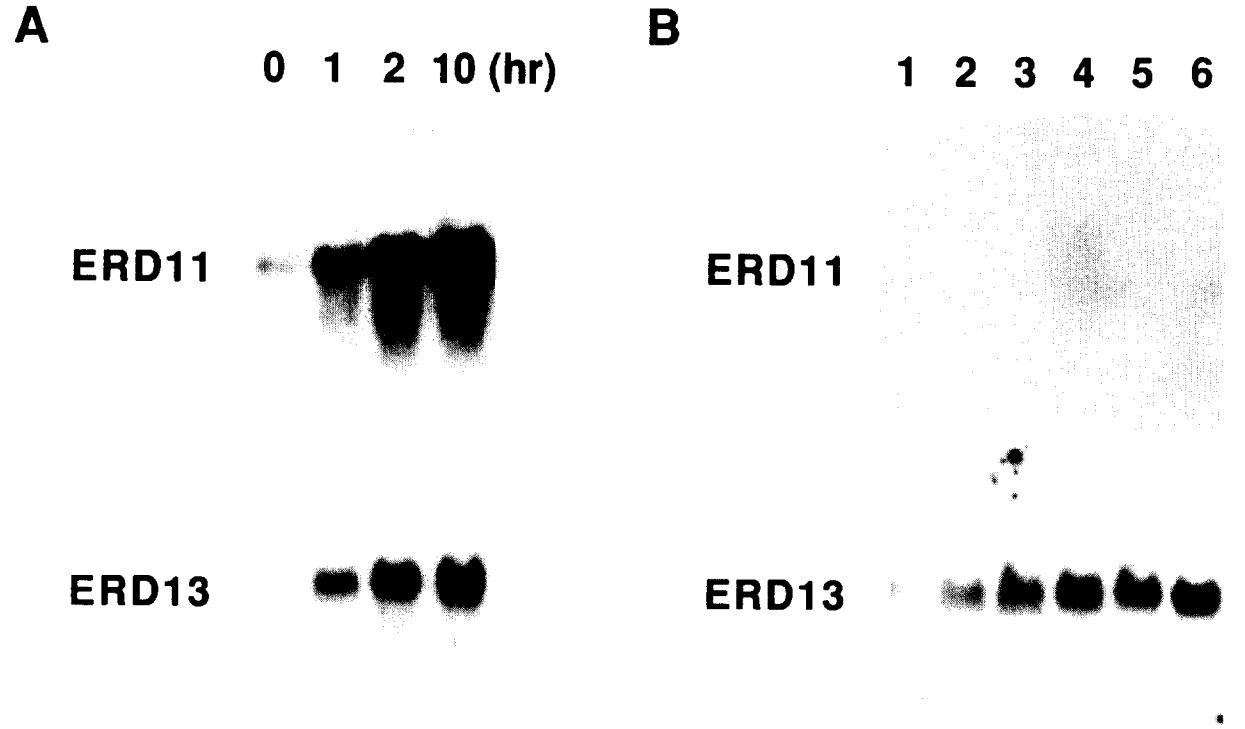

Fig. 3. Northern blot analysis of ERD11 and ERD13 gene-expression in dehydrated plants (A) and growth regulator treated plants (B) of *A. thaliana*. (A) One-month-old whole plants of *A. thaliana* were dehydrated for 0, 1, 2, and 10 h. Total RNA was extracted from these plants, fractionated on 1.2% agarose gels, blotted onto nitrocellulose membranes, and probed with ^{32}P-labelled cDNA fragments of ERD11 and ERD13. Ten μg of total RNA was loaded in each lane. (B) One-month-old whole plants of *A. thaliana*, grown axenically, (1) were transferred to liquid GM medium without (2) or with 10^{-4} M 2,4-D (3), BA (4), ABA (5), or GA3 (6), and incubated for 10 h. Total RNA was extracted from these plants, fractionated on 1.2% agarose gels, blotted onto nitrocellulose membranes, and probed with ^{32}P-labelled cDNA fragments of ERD11 and ERD13. Ten μg of total RNA was loaded in each lane.

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