

Nucleotide Sequence and Spatial Expression Pattern of a Drought- and Absciscic Acid-Induced Gene of Tomato¹

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ABSTRACT

The nucleotide sequence of *le16*, a tomato (*Lycopersicon esculentum* Mill.) gene induced by drought stress and regulated by abscisic acid specifically in aerial vegetative tissue, is presented. The single open reading frame contained within the gene has the capacity to encode a polypeptide of 12.7 kilodaltons and is interrupted by a small intron. The predicted polypeptide is rich in leucine, glycine, and alanine and has an isoelectric point of 8.7. The amino terminus is hydrophobic and characteristic of signal sequences that target polypeptides for export from the cytoplasm. There is homology (47.2% identity) between the amino terminus of the LE16 polypeptide and the corresponding amino terminal domain of the maize phospholipid transfer protein. *le16* was expressed in drought-stressed leaf, petiole, and stem tissue and to a much lower extent in the pericarp of mature green tomato fruit and developing seeds. No expression was detected in the pericarp of red fruit or in drought-stressed roots. Expression of *le16* was also induced in leaf tissue by a variety of other abiotic stresses including polyethylene glycol-mediated water deficit, salinity, cold stress, and heat stress. None of these stresses or direct applications of abscisic acid induced the expression of *le16* in the roots of the same plants. The unique expression characteristics of this gene indicates that novel regulatory mechanisms, in addition to endogenous abscisic acid, are involved in controlling gene expression.

During a period of drought stress, a plant undergoes a number of physiological and metabolic changes together with an increase in the biosynthesis of the plant hormone ABA (38). Because the accumulation of ABA is an early event after the onset of drought stress, it has been postulated that ABA acts to coordinate the plant's response to water deficit (16). These responses include the closure of stomata, resulting in a limitation of the transpiration rate, a decrease in the water potential of the plant, and decreased photosynthesis (2). Changes in gene expression have been observed after periods of water deficit as indicated by the accumulation of novel polypeptides (3, 12). In addition, a number of ABA-regulated genes have been isolated and characterized (for review, see ref. 32). Whereas the majority of these genes are expressed in embryos of developing seeds when the levels of ABA also rise, a few are also expressed in the wilting vegetative portions of the plant. Analysis of the 5'-flanking DNA sequences of two

genes expressed in developing seeds has revealed a putative ABRE² thought to be involved in the modulation of gene expression by ABA (22, 25, 37).

Earlier studies using the ABA-deficient mutant of tomato, *flacca*, demonstrated that ABA is involved in regulating some of the changes in gene expression that occur in response to drought stress (3). Subsequently, three cDNAs representing genes that are induced by drought stress and regulated by endogenous ABA were isolated and characterized (7). In this paper, the nucleotide sequence of the cDNA and corresponding genomic DNA fragment of one of these, pLE16, is presented. The spatial expression pattern and modulation of gene expression by other abiotic stresses were also investigated. The expression of this gene is unique, being specific to the vegetative, photosynthetic portions of the plant and negligible in developing seeds.

MATERIALS AND METHODS

Plant Material and Experimental Treatments

Tomato (*Lycopersicon esculentum* Mill. cv Ailsa Craig) was grown in the greenhouse for 4 to 6 months under natural daylength and well-watered conditions. Hydroponically grown plants were germinated in vermiculite and then maintained in an aerated solution of Stern's Miracle Gro for Tomatoes (according to the manufacturer's instructions) in a Western Environmental growth chamber (Napa, CA) for 3 to 4 weeks under a 12/12h day/night cycle, at 25.5°C, 70 to 75% RH, and 225 $\mu\text{E m}^{-2} \text{s}^{-1}$ light.

For drought stress treatments, leaves were harvested, wilted to 88% of their original fresh weight, and maintained in plastic bags (10 × 15 cm) for 6 h on the laboratory bench, after which they were frozen in liquid nitrogen and stored at -80°C. Drought-stressed stem and petiole tissue was collected from shoots that had been wilted to 88% of their original fresh weight, maintained in plastic bags, then frozen and stored as described above. Nonstressed stem and petiole tissue was harvested in the greenhouse directly into liquid nitrogen. Seeds were harvested from fruit at the following stages of development: MG 1 to 4, B, T, P, and R, described in Mitcham *et al.* (23). Seeds were frozen on dry ice before storage at -80°C. MG and R tomato fruit were harvested from the plant, the seeds were removed, and the pericarp tissue was frozen in liquid nitrogen. Individual stress treat-

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² Abbreviations: ABRE, abscisic acid response element; MG, mature green; B, breaker; T, turning; P, pink; R, red; PLTP, phospholipid transfer protein; kbp, kilobase pair; bp, base pair.

ments were conducted on hydroponically grown tomato plants by replacing the Miracle Gro hydroponic solution with PEG (to achieve a water potential of -1.4 MPa), NaCl (to 171 mM), or ABA (to 100 μ M) and exposing the plants for 6 h. Exposure to cold was achieved by placing plants at 4°C for 8 h, or to heat by placing at 40°C for 2 h. Drought stress treatments, on separated roots and shoots, were carried out as described above. Nonstressed tissue was collected from plants that had been removed from the Miracle-Gro solution and maintained in distilled water under hydroponic conditions for 6 h. After each experimental treatment, leaves or roots were weighed and frozen on dry ice for ABA determination or in liquid nitrogen for RNA extraction.

RNA Isolation and Blot Analyses

Total and poly(A)⁺ RNA was isolated as described by Cohen and Bray (7), except for the isolation of RNA from seeds in which an additional phenol:chloroform and ethanol precipitation was performed. Total RNA was size-fractionated in a formaldehyde denaturing 1.2% agarose gel according to Sambrook *et al.* (28). RNA was transferred to Zeta-probe (0.45 μ m; Bio-Rad) membranes using $20 \times \text{SSC}$ (3 M sodium chloride, 0.3 M sodium citrate, pH 7.0) as transfer buffer. After transfer, RNA was immobilized by cross-linking with uv irradiation at 300 nm for 3 min. Prehybridization and hybridization were carried out as described by Cohen and Bray (7) with the following modifications: both the prehybridization and hybridization were carried out at 20°C below the melting temperature, 0.5% SDS was added, and 3×10^7 cpm of an antisense RNA probe to pLE16 (the cDNA insert was subcloned into pBS-SK M13+, Stratagene, La Jolla, CA) was used (Promega Riboprobe System Technical Bulletin). Membranes were washed according to the manufacturer's instructions (Bio-Rad) except that each wash time was doubled and the final wash was repeated. To ensure that equal amounts of RNA were loaded, each blot was stained using the procedure outlined in Sambrook *et al.* (28).

Construction of a Tomato Genomic Library

Genomic DNA was isolated from tomato leaf tissue using the method described by Fisher and Goldberg (11). Genomic DNA was digested with *Bam*HI and sedimented in a 10 to 40% sucrose gradient in 1 M NaCl, 10 mM Tris-HCl (pH 7.5), 5 mM EDTA at $27,000$ rpm, 4°C for 24 h. DNA in the 9 to 23 kbp range was collected, ligated into prepared *Bam*HI λ GEM11 arms (Promega, Madison, WI) and packaged in a Gigapack plus packaging extract (Stratagene). Recombinants were grown on the permissive host strain KW251 (Promega). The resulting plaque lifts were screened using pLE16, radio-labeled by the random oligonucleotide priming method (10). Positive recombinant phage was purified and the insert subcloned into pGem1 (Promega) to produce the recombinant plasmid, pGLE16.

Determination of Nucleotide Sequence

A 3.0 kbp *Hind*III fragment containing the gene for *le16* was subcloned from pGLE16 into pBS-SK M13+. Ordered

unidirectional deletions were constructed from both ends of the genomic *Hind*III fragment and the cDNA insert of pLE16 as described by Henikoff (17) using exonuclease III (Promega) and mung bean nuclease (Pharmacia, Piscataway, NJ). The nucleotide sequence of the deletions was determined by the dideoxy chain termination method of Sanger *et al.* (29) using the LKB ³²P Sequencing kit (Pharmacia) and [³²S] dATP (45 TBq mmol⁻¹; New England Nuclear). DNA and amino acid sequence analyses were performed using the Wisconsin Genetics Computer Group sequence analysis package (8). For deduced amino acid sequence comparisons, the degree of similarity was predicted using the Dayhoff table (30). The hydropathy plot was generated using the method of Kyte and Doolittle (20).

ABA Quantification

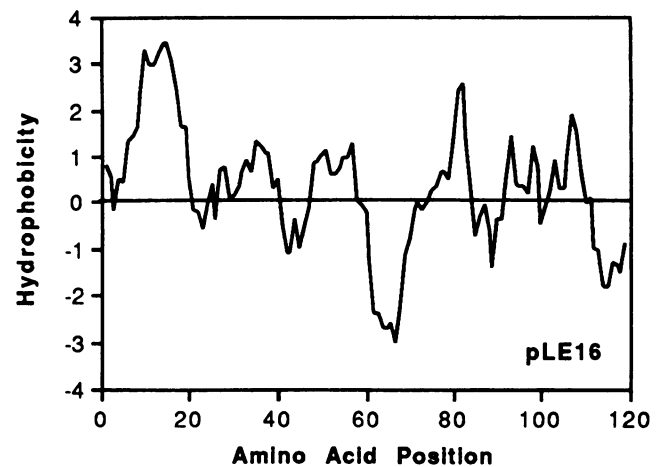
ABA was quantified using a competitive ABA radio-immunoassay as described by Bray and Beachy (4).

RESULTS

Nucleotide and Deduced Amino Acid Sequence of the cDNA pLE16

The nucleotide sequence of the cDNA pLE16 was determined and is shown in Figure 1 (bold-face type), together with the deduced amino acid sequence. Assuming that initiation of translation occurs at the first Met, the gene contains an open reading frame of 119 amino acids, capable of encoding a polypeptide with a predicted molecular mass of $12,717$ D and isoelectric point of 8.7 . Leu (15.0%), Gly (11.0%), and Ala (8.4%) are the most abundant amino acids present in the deduced polypeptide, which is deficient in Trp. The hydropathy plot presented in Figure 2 shows both hydrophilic and hydrophobic regions throughout the polypeptide. Overall, the protein is hydrophobic, with a large hydrophilic region between amino acids 59 to 74 and another at the carboxy terminus. The most hydrophobic region of the protein is at the amino terminus (residues 1 – 24). The amino acid sequence corresponding to this domain shares characteristics of signal sequences that include a charged amino terminal region, a central hydrophobic core, and a polar carboxy terminal region (36). Cleavage of this putative signal sequence is predicted to occur after the first Ser residue (residue 24 after the first Met), based on the rules outlined by Von Heijne (35). The amino terminal portion of the protein, corresponding to amino acids 25 to 70 , contains the helix-breaking amino acids Gly and Pro frequently spaced, suggesting that this portion of the protein does not form an extensive α or β helix structure. The carboxy terminal 71 to 119 amino acids contains fewer of these amino acids, indicating the potential ability to form α helices.

A search of the National Biomedical Research Foundation protein database release 25.0 revealed some similarity between the polypeptide encoded by *le16* and the maize PLTP (33). This is shown in Figure 3, where the amino acid sequence for LE16 is aligned with that for the maize PLTP. The similarity is greatest at the amino terminal domains of the mature polypeptides (Fig. 3, between the vertical arrows), with 47.2% identity and 88.8% similarity. Thereafter, the similarity be-



pletely sequenced. Figure 1 shows the nucleotide sequence of the DNA fragment containing *le16*. The nucleotide sequence of both the cDNA and genomic clone are identical for the coding as well as the 5' and 3' untranslated regions (Fig. 1). This indicates that the isolated gene corresponds to the cDNA pLE16, characterized by Cohen and Bray (7). Southern genomic hybridization analysis indicated that the gene for *le16* is present in the genome as a single copy (5). The 1974 bp promoter sequence presented in Figure 1 is AT-rich and contains a TATAA and CAAT box (Fig. 1, italicized), common to eukaryotic genes. The transcription start site was determined by primer extension (data not shown) and mapped to nucleotide +1, 28 bp downstream of the TATAA box (Fig. 1). The T/CACGTGGC ABRE or GC-rich sequence motifs, present in the 5'-flanking DNA sequences of several ABA-responsive genes (22, 25), were not found in the 5'-flanking DNA sequences of *le16*. However, the sequence motif CACGTAAC, which has some similarity to the consen-

	Le	---mEmFGkIACfVVFcMvvVAPhAES	24
	Zm	martQqLAvVATaVvAlVLLAAAtSEA	27
		▼ Le ▼ ▼ Zm ▼	
Le	-LSCGEVTSGLAPCLPYI--EGRGP1GGCCGvKGL1GARpKqTgRQRAlAl	74	
Zm	AISCGQVASAIAPCISYargQSGSPsAGCCSGVRSLNNAARtTaDrRAaCnCL	80	
Le	NRQl1LRAL-----IQEKPIGSLVESfTf1TrSaPLiaqrsskvdcs	119	
Zm	KNAaagVSGLnagnaasIPKSGQVSP1PYTISTdSCrVn	120	

Figure 3. Amino acid comparison of the deduced polypeptide of *le16* with the maize PLTP. LE16 (*Le*, *L. esculentum*) amino acid sequence is aligned with the amino acid sequence of maize PLTP (*Zm*, *Zea mays*). Bold-face, capital letters indicate amino acid identity; capital letters indicate amino acids with a similarity of >0.1 . Similarities were predicted using the Dayhoff table (30). Lower case letters indicate no similarity. The numbering on the right indicates the amino acid residue number. *Le* amino acid residues 1 to 24 and *Zm* amino acid residues 1 to 27 represent the putative amino terminal signal peptide sequences. The sequence between the vertical arrows represents the domain of highest amino acid identity between the polypeptides.

A tomato genomic library was screened with the cDNA pLE16 and its corresponding genomic fragment was isolated and subcloned to generate the plasmid pGLE16. From this clone, a 3.0 kbp *Hind*III fragment was subcloned and com-

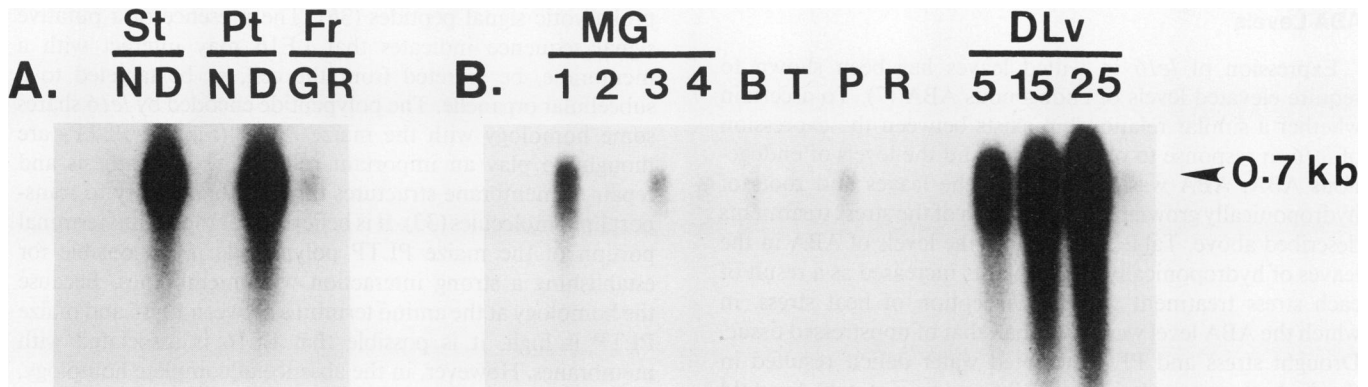


Figure 4. RNA blot analysis of *le16* transcript accumulation in different tissues of the plant. A, Thirty micrograms of total RNA isolated from drought-stressed (D) and nonstressed (N) stem (St), petiole (Pt), and mature green (G) and red (R) fruit (Fr) pericarp tissue was size-fractionated in a formaldehyde agarose gel, blotted, and hybridized with an antisense RNA probe to pLE16. B, Twenty-five micrograms of total RNA isolated from seeds of developing fruit (MG 1, 2, 3, 4 and B, T, P, and R) together with 5, 15, and 25 µg (5, 15, 25) of drought-stressed leaf RNA (DLV) were treated as described above.

sus ABRE, lies at -135 relative to the start of transcription (Fig. 1, underlined). An AT-rich intron of 157 bp interrupts the gene close to the 3' end and is flanked by the consensus GT and AG border dinucleotides in the genomic sequence. The 450 bp 3' noncoding region contains a putative polyadenylation signal AATGAA (Fig. 1, bold-face type and underlined), which has been described for other plant genes (27).

Spatial Expression of *le16*

Many ABA-induced genes expressed in developing seeds are also expressed in wilted vegetative tissue (32). pLE16 was isolated from a drought-induced tomato leaf cDNA library; therefore, it was of interest to determine in what additional tissues of the plant *le16* is expressed. To this end, whole cell RNA was isolated from drought-stressed and nonstressed stems, petioles, and roots, MG and R fruit pericarp, and seeds extracted from fruit at the MG 1 to 4, B, T, P, and R stages of development. This RNA was used for RNA blot hybridization analyses together with RNA isolated from nonstressed and drought-stressed leaf tissue. A high level of RNA corresponding to *le16* accumulated in drought-stressed leaves, stems, and petioles (Figs. 4 and 5). A much lower level of *le16* RNA accumulated in MG fruit, nonstressed leaves, and developing seeds. To detect the *le16* transcript, the seed blot was exposed 16-fold longer; the inclusion of drought-stressed leaf RNA samples on the same blot serves to illustrate the low level of *le16* expression in the developing seeds. No accumulation of RNA for *le16* was detected in the pericarp of R fruit, drought-stressed roots, or nonstressed stems, petioles, and roots (Figs. 4A and 5).

Expression of *le16* in Response to Other Stress Treatments

To determine if *le16* is expressed in response to a range of abiotic stresses, hydroponically grown plants were exposed to the following treatments: drought stress, PEG-mediated water deficit, salinity, cold stress, heat shock, and exogenous applications of ABA. Whole cell RNA was isolated from the leaves

and roots of nonstressed and treated plants and used for RNA blot hybridization analyses. Figure 5 shows that RNA for *le16* accumulated in leaf tissue in response to each of the stresses tested. The accumulation of RNA was greatest in leaves of drought-stressed plants. RNA also accumulated, although to a lesser extent, in the leaves of PEG-, salt-, cold-, and ABA-treated plants and to a very low level in leaves of heat-stressed plants. These results were verified by densitometer scans of the original autoradiogram. For drought-stressed leaves, there was a 127-fold increase of *le16* RNA over the nonstressed RNA level, and a 4.2-, 4.6-, 3.1-, 1.4-, and 7.3-fold increase over the nonstressed RNA level for PEG-, salt-, cold-, heat-, and ABA-treated plants, respectively. As previously observed (7), a low level of *le16* expression was present in nonstressed leaves (Fig. 5). No expression of *le16* was observed in root tissue, regardless of the imposed stress, including ABA application.

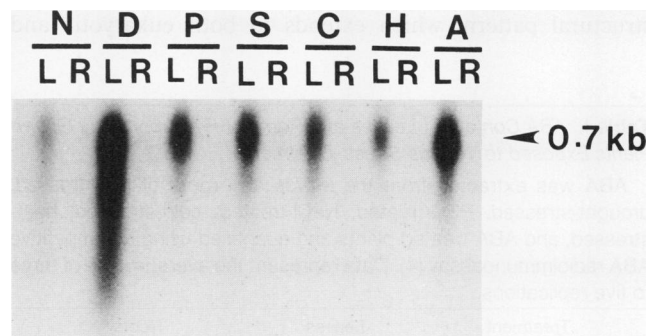


Figure 5. RNA blot analysis of *le16* transcript accumulation in the leaves and roots of hydroponically grown tomato plants exposed to various abiotic stresses. Thirty micrograms of total RNA isolated from the leaves (L) and roots (R) of nonstressed (N), drought-stressed (D), PEG-treated (P), NaCl-treated (S), cold-stressed (C), heat-stressed (H), and ABA-treated (A) plants was size-fractionated in a formaldehyde 1.2% agarose gel, blotted, and hybridized to an antisense RNA probe to pLE16.

ABA Levels

Expression of *le16* in wilted leaves has been shown to require elevated levels of endogenous ABA (7). To ascertain whether a similar relationship exists between the expression of *le16* in response to other stresses and the levels of endogenous ABA, ABA was measured in the leaves and roots of hydroponically grown plants after each of the stress treatments described above. Table I shows that the levels of ABA in the leaves of hydroponically grown plants increased as a result of each stress treatment with the exception of heat stress, in which the ABA level was lower than that of nonstressed tissue. Drought stress and PEG-mediated water deficit resulted in the highest accumulation of ABA, approximately fourfold over the nonstressed level, whereas NaCl- and ABA-treatments caused approximately a threefold and cold stress a twofold increase. Overall, the ABA levels of the roots were lower than those of the leaves. After each of the stress treatments, only drought stress, PEG-mediated water deficit, and direct ABA applications resulted in root ABA levels greater than those of nonstressed plants. Direct ABA applications resulted in a 113-fold increase in ABA levels, drought a 3.4-fold increase, and PEG-mediated water deficit a 1.3-fold increase. Salinity, cold, and heat stress resulted in a lower root ABA level relative to the roots of nonstressed plants.

DISCUSSION

le16, a gene whose expression in detached drought-stressed tomato leaves has been shown to require elevated levels of endogenous ABA (7), is described. Analysis of the nucleotide sequence of *le16* (Fig. 1) revealed the presence of an open reading frame capable of encoding a polypeptide of 12.7 kD. The hydropathy plot (Fig. 2) further indicated that the protein contains both hydrophilic and hydrophobic domains, in contrast to many of the proteins induced by drought and ABA, which tend to be overwhelmingly hydrophilic (9). The domain of greatest hydrophobicity occurs at the amino terminus, and the amino acid sequence of this domain has characteristics common to signal peptides. Whereas signal sequences show little homology at the amino acid level, all share a common structural pattern, which extends to both eukaryotic and

prokaryotic signal peptides (36). The presence of a putative signal sequence indicates that LE16 may interact with a membrane, be secreted from the cell, or be targeted to a subcellular organelle. The polypeptide encoded by *le16* shares some homology with the maize PLTP (Fig. 3). PLTPs are thought to play an important role in the biosynthesis and repair of membrane structures through their ability to transport lipid molecules (33). It is believed that the amino terminal portion of the maize PLTP polypeptide is responsible for establishing a strong interaction with membranes. Because the homology at the amino terminus between LE16 and maize PLTP is high, it is possible that LE16 is associated with membranes. However, in the absence of complete homology, LE16 is unlikely to function exactly as the maize PLTP.

The spatial expression pattern of *le16* is unique, with expression being confined to the aerial, photosynthetic portions of the plant. Expression was observed predominantly in drought-stressed leaves, stems, and petioles, whereas a low level of expression was detected in the pericarp of MG fruit and developing seeds (Figs. 4 and 5). However, as shown in Figure 5, it should be noted that a low level of *le16* gene expression has consistently been observed in nonstressed leaf tissue (7). The expression observed in the MG fruit and seeds may result from this constitutive expression. This pattern of gene expression contrasts with that of many of the previously described drought- and ABA-regulated genes that are expressed in leaves, roots, and seeds (32, 37).

In addition to being induced by drought stress, expression of *le16* was induced in leaves of hydroponically grown plants by other abiotic stresses, including PEG-mediated water deficit, salinity, cold stress, and heat stress (Fig. 5). PEG-mediated water deficit, salinity, and cold stress each have a water loss component; therefore, the expression of *le16* in response to these stresses is not surprising. Changes in gene expression in response to salinity have been observed and the same salt-induced genes are also induced by drought stress, although to a lesser extent (6, 18, 31). A similar situation exists for cold stress, with some cold-induced genes also being induced by drought stress (15, 19). Furthermore, the cold stress induction of *rab 16*, a drought- and ABA-regulated gene family, has been observed in rice (14). Each of the abiotic stresses tested, with the exception of heat shock, resulted in an increase in the level of endogenous ABA in the leaf tissue (Table I). Therefore, excepting heat stress, the stress-induced expression of *le16* was coincident with elevated levels of ABA (Table I, Fig. 5). As such, expression of *le16* in the leaves of hydroponically grown plants exposed to several abiotic stresses may be regulated by ABA. Indeed, some of the observed changes in gene expression in response to salinity and cold stress are believed to be ABA regulated (6, 15, 18, 19, 31). However, the accumulation of *le16* RNA without a corresponding ABA increase in the leaves of heat-stressed plants (Table I, Fig. 5) indicates that elevated levels of ABA do not regulate the expression of *le16* to all stresses.

Although induction of *le16* expression was observed together with elevated ABA levels in the leaves of stressed plants, no such relationship was observed in the roots and seeds. Endogenous ABA levels increased in roots of plants subjected to drought stress, PEG-mediated water deficit, and exogenous ABA (Table I). Expression of *le16*, however, was not observed

Table I. ABA Content of Leaves and Roots of Hydroponically Grown Plants Exposed to Various Stress Treatments

ABA was extracted from the leaves and roots of nonstressed, drought-stressed, PEG-treated, NaCl-treated, cold-stressed, heat-stressed, and ABA-treated plants and quantified using a competitive ABA radioimmunoassay (4). Data represent the average $\pm$ SE of three to five replications.

Treatment	Leaves	Roots
	ng ABA/g fresh wt	
Nonstress	222.8 $\pm$ 14.7	72.8 $\pm$ 63.8
Drought stress	942.1 $\pm$ 128.4	246.7 $\pm$ 119.0
PEG	827.3 $\pm$ 174.2	93.8 $\pm$ 14.1
Salt stress	615.4 $\pm$ 48.0	37.1 $\pm$ 6.4
Cold stress	463.6 $\pm$ 42.7	29.1 $\pm$ 1.6
Heat stress	158.2 $\pm$ 16.4	11.0 $\pm$ 0.3
ABA treatment	618.0 $\pm$ 46.7	8251.6 $\pm$ 633.1

in root tissue, regardless of the stress treatment or the endogenous ABA content (Fig. 5, Table I). The ABA levels of the developing seeds were high, increasing from the MG 1 to B stages (A. Cohen and E. A. Bray, personal communication), yet the accumulation of RNA corresponding to *le16* was low in seeds (Fig. 4B). Differential sensitivity of *le16* to ABA may explain these observations and the tissue-specific expression of this gene. As such, induction of *le16* expression by ABA would only occur at the relatively low levels observed in drought-stressed leaves and not at the higher levels found in the developing seeds, which may actually inhibit gene expression. Indeed, when varying amounts of ABA were administered to detached leaves, optimal expression of *le16* occurred at 100 μ M, whereas 1 mM resulted in a reduced accumulation of *le16* RNA (7). Furthermore, it is also possible that induction of *le16* expression by ABA in the vegetative aerial tissue may be dependent upon signals not present in the roots or seeds. Conversely, a repressor of gene induction, present in the roots and seeds, may be absent or deactivated in the shoot tissue. Others have observed differential responses to ABA. In the case of the proteinase inhibitor II genes, systemic induction by ABA was observed, yet during drought stress, when the levels of endogenous ABA presumably rise, there was no induction of gene expression (26).

Elements involved in ABA regulation of gene expression have been described in two ABA- and drought-induced genes: *rab 16A* in rice (24) and *Em* in wheat (21). Sequence-specific binding of a protein factor present in nuclear extracts to 5'-flanking DNA containing the sequences T/CACGTGGC and cCGcCGCGCTg has been observed (13, 25), and recently a cDNA clone corresponding to a DNA binding protein that interacts with the first of these sequences in the *Em* gene was isolated (13). These ABREs are present in the promoters of a number of other ABA- and drought-induced genes, which include *rab 16B-D* (38) and *RAB-17* (34); they are also present in the 5'-flanking DNA of the late embryogenesis abundant (*lea*) genes from cotton, with the exception of *gD11* (1). The *le16* sequence motif with the greatest homology to the ABRE, CACGTAAC, lies at -135 relative to the start of transcription (Fig. 1). Whether this element, or others, is involved in the induction of *le16* gene expression by ABA awaits functional analysis of the 5'-flanking DNA sequences. To date, the functional analysis of drought- and ABA-induced genes has been confined to those that are expressed in seeds during the late stages of development, although both the *rab* gene family and *Em* are also induced in shoots by ABA and osmotic stress (22, 37). The elucidation of the sequences responsible for ABA and drought induction of *le16* gene expression will be of interest because this gene has been shown to be regulated by endogenous ABA and is expressed specifically in the photosynthetic vegetative tissue in response to a wide range of environmental signals.

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