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Organ-Specific and Environmentally Regulated Expression of Two Abscisic Acid-Induced Genes of Tomato[1]

Nucleotide Sequence and Analysis of the Corresponding cDNAs

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ABSTRACT

The cDNAs, pLE4 and pLE25, represent mRNAs that accumulate in response to water deficit and elevated levels of endogenous abscisic acid in detached leaves of drought-stressed tomato (*Lycopersicon esculentum* Mill., cv Ailsa Craig) (A Cohen, EA Bray [1990] Planta 182: 27–33). DNA sequence analysis of pLE4 and pLE25 showed that the deduced polypeptides were 13.9 and 9.3 kilodaltons, respectively. Each polypeptide was hydrophilic, cysteine- and tryptophan-free, and found to be similar to previously identified proteins that accumulate during the late stages of embryogenesis. pLE4 and pLE25 mRNA accumulated in a similar organ-specific pattern in response to specific abiotic stresses. Yet, expression patterns of the corresponding genes in response to developmental cues were not similar. pLE25 mRNA accumulated to much higher levels in developing seeds than in drought-stressed vegetative organs. pLE4 mRNA accumulated predominantly in drought-stressed leaves. The similarities and differences in the accumulation characteristics of these two mRNAs indicates that more than one mechanism exists for the regulation of their corresponding genes.

Water deficit results in numerous metabolic, developmental, and physiological changes within a plant (19, 33, 42), including changes in gene expression. The accumulation of specific mRNAs in response to water deficit has been described in tomato leaves (10), pea shoots (17), maize and barley seedlings (9, 16, 31), and the leaves and roots of the resurrection plant, *Craterostigma plantagineum* (30). Water deficit also results in an increase in the concentration of the plant hormone ABA (42). Increases in endogenous ABA levels have been shown to correspond with the accumulation of several novel mRNAs and proteins in wilted vegetative tissues (3, 4, 9, 10, 16). Furthermore, studies involving ABA-deficient mutants have demonstrated that their inability to accumulate water deficit-induced gene products is restored through ABA applications (4, 10, 31). Although most gene expression studies to date cannot confirm endogenous ABA as a regulator of gene expression during periods of water deficit, they have shown that water deficit-induced genes are expressed in response to exogenous ABA (4, 7, 10, 30, 40, 41). Therefore, it appears that ABA plays an important role in a plant's ability to regulate changes in gene expression in response to water loss.

Many of the genes expressed in wilted vegetative tissues are also expressed in seeds during the late stages of embryogenesis (T.J. Close, personal communication; 16, 20, 29, 31, 41). These genes, and others expressed late in seed development, can be precociously induced in embryos treated with exogenous ABA (14, 16, 20, 31). Several homology groups have been identified for these ABA-responsive genes based on similarities at the nucleotide and deduced amino acid levels (12, 38). Conserved domains among these genes, in addition to common expression characteristics in both vegetative and embryonic tissues, suggest that their encoded polypeptides may play a role in protecting plant tissues during periods of water loss.

In addition to exogenous ABA applications, salinity and cold stress also result in the expression of many genes induced by water loss (7, 15, 18, 28–30). This is not surprising because water deficit, salinity, and cold stress each have an osmotic stress component that results in reduced cell water potential (1, 25). Salt and cold stress also result in elevated endogenous ABA levels (28, 39), although generally lower than the levels observed after water deficit. Therefore, ABA may be a common mediator for the induction of gene expression in response to environmental stress. However, water deficit- and cold-induced mRNAs and proteins that are not ABA responsive have also been identified (17, 27), indicating that more than one mechanism for the induction of osmotic stress-induced genes must exist.

In an earlier study, we identified several cDNAs representing mRNAs that accumulate in detached tomato leaves in response to water deficit and elevated levels of ABA (10). The present work describes the nucleotide sequence of two cDNAs, pLE4 and pLE25, and a comparison of their deduced amino acid sequences with other previously described polypeptides. Characterization of pLE4 and pLE25 mRNA accumulation in different organs during plant development and in response to a number of abiotic stresses was performed to further our understanding of the mechanism by which ABA regulates gene expression.

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MATERIALS AND METHODS

Plant Material and Experimental Treatments

Tomato, *Lycopersicon esculentum* Mill., cv Ailsa Craig (obtained from Dr. J.W. Maxon Smith at the Glasshouse Crops Research Institute in Littlehampton, West Sussex, England), plants were grown under standard greenhouse conditions for 2 to 4 months or in hydroponics for 3 weeks. 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) (12/12 h day/night cycle, 25°C, 70–75% RH, and 225 μE m^{-2} s^{-1} of light).

Nonstressed petioles and stems were removed from greenhouse-grown plants and immediately frozen in liquid nitrogen. Tissues used for drought-stress treatments were wilted to 88% of their original fresh weight on the laboratory bench and then placed in plastic bags for 6 h prior to freezing (10). Hydroponically grown tomato plants were exposed to six individual treatments: PEG, NaCl, ABA, cold stress, heat stress, and drought stress. The Miracle Gro hydroponic solution in which the plants had been maintained was replaced with distilled water during each treatment period. PEG or NaCl was added to achieve a water potential of −1.4 MPa or 171 mM, respectively. ABA was added to a concentration of 100 μM. Plants were maintained in these solutions for 6 h. Cold-treated plants were taken from 25°C and placed at 4°C for an 8-h period, whereas heat-stressed plants were placed at 40°C for 2 h. A 6 h drought stress was achieved by removing whole plants from the hydroponic conditions, separating the shoots from the roots, and allowing the tissues to dry to 88% of their original fresh weight as described by Cohen and Bray (10). Nonstressed plants were kept in distilled water for 6 h. At the completion of each treatment period, leaflets or roots were weighed and placed on dry ice for ABA extraction, or frozen in liquid nitrogen for RNA extraction. All tissues were stored at −80°C.

Using greenhouse-grown tomato plants, seeds were harvested from fruit at eight stages of development as described by Mitcham *et al.* (26), MG1[2] to 4, B, T, P, and R, and placed on dry ice. MG and R fruit pericarp was frozen in liquid nitrogen. All tissues were stored at −80°C.

RNA Isolation and Analyses

Total RNA was extracted from tomato leaflets, petioles, stems, and fruit using the LiCl-phenol extraction method described by Prescott and Martin (32). RNA was extracted from seeds using this procedure with an additional phenol:chloroform (1:1, v/v) extraction and ethanol precipitation.

RNA was separated by size on formaldehyde denaturing 1.2% agarose gels according to Sambrook *et al.* (35). RNA was transferred to Zeta-Probe (0.45 μm; Bio-Rad) membranes using 20 × SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0) as a transfer medium. RNA was cross-linked to the membranes by exposure to transmitted UV irradiation of 300 nm for 3 min. Prehybridizations and hybridizations were carried out according to Cohen and Bray (10) with the following modifications. Both the prehybridization and hybridization temperatures were adjusted to T_m −20°C for each cDNA insert, and SDS to 0.5% was added to the prehybridization and hybridization buffers. All membranes were hybridized with 2.5 to 5.0 × 10^6 cpm/mL of an antisense RNA probe (Promega Riboprobe System Technical Bulletin). Membranes were washed under the following conditions: 2 × SSC/0.2% SDS at room temperature for 5 min followed by two 20 min washes; 1 × SSC/0.5% SDS at 65°C for 30 min two times; and 0.1 × SSC/0.5% SDS at 65°C twice for 30 min followed by a 1 h wash. All RNA isolations and blots were replicated at least two times. To ensure that an equal quantity of RNA from each sample was transferred, membranes were stained for ribosomal RNAs (35).

ABA Quantification

ABA quantification was completed on three to eight replications using a competitive ABA radioimmunoassay as described by Bray and Beachy (5).

Determination of Seed Fresh and Dry Weight

Seed fresh weight was determined for individual seeds of mature tomato fruit, MG1 to R. Seed dry weight was determined after oven drying at 105°C overnight.

Determination of Nucleotide Sequence and Analysis

The pLE4 cDNA insert was subcloned from the pGEM-1 vector (Promega, Madison, WI) into the pBS-SK vector (Stratagene, La Jolla, CA). The DNA sequence of both strands of the pLE4 and pLE25 cDNA inserts was determined using the Pharmacia LKB ^{T7}Sequencing Kit (Pharmacia) and [^{35}S] dATP (45.0 TBq $mmol^{-1}$; New England Nuclear) (36). An exonuclease III deletion series (21) was used to produce unidirectional deletions within the cDNA inserts. Mung bean nuclease (Pharmacia) was used in place of S1 nuclease to remove the undigested single strands that were generated by the exonuclease III digestions. Nucleotide and amino acid analyses were performed using the Genetics Computer Group sequence analysis software package (11). For the deduced polypeptide comparisons, the degree of similarity between amino acids was determined using the Dayhoff table (37). Hydropathy plots were generated using the method of Kyte and Doolittle (24).

RESULTS

DNA and Predicted Amino Acid Sequences of the cDNAs pLE4 and pLE25

The pLE4 cDNA is 856 base pairs in length and contains an open reading frame of 130 amino acids, beginning at the first Met codon (Fig. 1A). The context surrounding the first Met (TCAAAGATGGCA) matches the translational initiation consensus sequence (TAAACAATGGCT) at the −6, −4, −3, +4, and +5 positions (23). A putative polyadenylation

[2] Abbreviations: MG1 to 4, mature green stages 1 to 4; B, breaker; T, turning; P, pink; R, red.

A. pLE4

```
-108                                                    GACTCTAGATTTTTTTTTTTTTTTTTTA
 -79  TAGCACAAGTTATATTATTTCAAAAACAAAGTAAAAAATTAAACAGTGTTTTTTAAAAAAAAAGAATTTTTGATCAAAG
   1  ATG GCA CAA TAC GGC AAT CAA GAC CAA ATG CGC AAG ACT GAT GAA TAT GGA AAC CAT GTC
 (1)  Met Ala Gln Tyr Gly Asn Gln Asp Gln Met Arg Lys Thr Asp Glu Tyr Gly Asn His Val
  61  CAA GAA ACA GGA GTC TAT CAA GGT ACC GGT ACT GGC GGT ATG ATG GGG GGC ACG GGT ACT
 (21)  Gln Glu Thr Gly Val Tyr Gln Gly Thr Gly Thr Gly Gly Met Met Gly Gly Thr Gly Thr
 121  GGC GGT ATG ATG GGG GGC ACT GGT GGA GAA TAT GGA ACT CAA GGC ATG GGT ACT GGT ACT
 (41)  Gly Gly Met Met Gly Gly Thr Gly Gly Glu Tyr Gly Thr Gln Gly Met Gly Thr Gly Thr
 181  CAT CAC CAT GAG GGG CAA CAG CAG CTT CGT CGA TCC GAC AGC TCT AGC TCG TCG GAG GAT
 (61)  His His His Glu Gly Gln Gln Gln Leu Arg Arg Ser Asp Ser Ser Ser Ser Ser Glu Asp
 241  GAT GGA GAA GGT GGG AGG AGA AAG AAG GGT TTG AAG GAG AAG ATA ATG GAG AAG ATG CCT
 (81)  Asp Gly Glu Gly Gly Arg Arg Lys Lys Gly Leu Lys Glu Lys Ile Met Glu Lys Met Pro
 301  GGA CAA CAT GAA GGT GAG TAT GGA CAA ACA ACA GGT GAA GAG AAG AAA GGA ATG ATG GAC
(101)  Gly Gln His Glu Gly Glu Tyr Gly Gln Thr Thr Gly Glu Glu Lys Lys Gly Met Met Asp
 361  AAA ATC AAG GAC AAG ATC CCT GGG ATG CAT TGA ACACCTTTGTTTTCATCTCCATCTTATCTTATGAA
(121)  Lys Ile Lys Asp Lys Ile Pro Gly Met His *
 429  TAAATAAGGTAGTGCTTGATTCTATTTCATGCACTAATTAGTAGTAATCGTTATGCCAGTAATTATCTAATTACGTACA
 508  CTCTTGTGTTTAAAGTCGTGTAAAGTGTGCTGACGCTATATACATGTGTGTGTATATTGGCGGCCATGCCCTGCCCCTT
 587  CTGTATTTTTAAAACAAGAATATTGCTTCCATGCTTGGAAAGCAATGATCATGTTGATGTGTGTTATGGTGTCCCGTGT
 666  TTAATGATGTTTTGGAAATAATATAACTTGTGCTATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 745  AAAA
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B. pLE25

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 -54                                                AAGAAGAAGAGAAGAAAACGAATTATATTGATTTAAGTTAAGAAAAACAGAAAA
   1  ATG CAG ACA GGA AAG GAC GCA GCA TCT GCA GCC AAA GCT GGC ATG GAG AAA ACC AAA GCC
 (1)  Met Gln Thr Gly Lys Asp Ala Ala Ser Ala Ala Lys Ala Gly Met Glu Lys Thr Lys Ala
  61  AAT GTT CAA GAG AAG GCG GAG AGG ATG ACG ACG AGG GAT CCG TTG AAG AAA GAG ATG GCG
 (21)  Asn Val Gln Glu Lys Ala Glu Arg Met Thr Thr Arg Asp Pro Leu Lys Lys Glu Met Ala
 121  ACG GAG AAG AAA GAG GAT AGG GTT GCA GCG GCG GAG ATG GGT AAG AGA GAT GCA AAA GCA
 (41)  Thr Glu Lys Lys Glu Asp Arg Val Ala Ala Ala Glu Met Gly Lys Arg Asp Ala Lys Ala
 181  CAA CAT GCA GCT GAA AAA CAA GGA GCT GCT ACC ACT GGA ACT GGA ACT ACT GGT TAT GGT
 (61)  Gln His Ala Ala Glu Lys Gln Gly Ala Ala Thr Thr Gly Thr Gly Thr Thr Gly Tyr Gly
 241  GCC ACT GGC AAT CAT ACA ACT TTC TAG AAGAAAAAAGGAGAAATGAAGCTAGTTGCTTTTAATTTTGAT
 (81)  Ala Thr Gly Asn His Thr Thr Phe *
 311  TTTCTCTTTTTTTTTTCTTTTTTTTCTTTTTGCTTTGTTATATGGGAAGTGATGTGATGACACACTTTTGTGTGGGTGT
 390  TGGCTTTTAAGATTTGACACCAAATTAAATAAATAAATGAAGTGGTAACTCTTCCTATAAAAAAAAAAAAAAAAAAAA
 469  AAAAAAAAAAAAAA
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Figure 1. Nucleotide and deduced amino acid sequences of the cDNAs pLE4 (A) and pLE25 (B). Nucleotide +1 was assigned to the predicted translation initiation codon, and the negative numbers refer to the 5′-untranslated regions. The predicted amino acids, shown in the three letter code, are numbered and in parentheses. Each stop codon is marked with an asterisk and putative polyadenylation sites are underlined. A line is drawn above the corresponding nucleotide sequence of the nine amino acid direct repeat in the LE4 polypeptide.

site (AATAA) is located 15 nucleotides upstream of the poly(A) tail. The open reading frame of pLE4 encodes a deduced polypeptide of 13,948 D with the compositional bias for the amino acids Gly (22.8%), Glu (9.2%), Met (9.2%), Thr (9.2%), and Gln (8.5%). The amino acids Cys, Phe, and Trp are not present in this polypeptide. A tandem repeat of nine amino acids Gly-Thr-Gly-Gly-Met-Met-Gly-Gly-Thr is present 30 amino acids downstream from the proposed start of translation. A hydropathy plot of the deduced amino acid sequence shows that the predicted LE4 polypeptide is largely hydrophilic (Fig. 2A).

The pLE25 cDNA is 539 base pairs in length (Fig. 1B). Two overlapping putative polyadenylation sites (AATAAA) are located approximately 22 nucleotides upstream of the poly(A) tail. An open reading frame of 88 amino acid residues, beginning at the first Met codon, encodes a deduced polypeptide of 9258 D. The context around the first Met (AGAAAA ATGCAG) matches the translational initiation consensus sequence (23) at only the −4, −3 and −1 positions. The deduced polypeptide has an amino acid composition rich in Ala (20.5%), Thr (14.8%), Lys (13.6%), and Gly (10.2%). Similar to LE4, LE25 lacks the amino acids Cys and Trp, in addition to Ile. A hydropathy plot (Fig. 2B) shows that this deduced polypeptide is overwhelmingly hydrophilic based on its predicted amino acid sequence.

Sequence Comparison of the LE4 and LE25 Polypeptides with Previously Described Proteins

The deduced amino acid sequences of pLE4 and pLE25 are each similar to previously identified proteins (Fig. 3). The LE4 polypeptide is similar to proteins of the DHN family in barley and maize (8, 9), the RAB family in maize (40) and rice (41), two proteins in the *C. plantagineum* family (30), and the cotton D11 (2, 12) and radish RSLEA2 (34) proteins. Figure 3A shows a comparison between the LE4 polypeptide (Le) and that of RAB-17 (Zm; 40), RAB 16A (Os; 41), DHN4 (Hv; 8, 9), pcC6-19 (Cp; 30), RSLEA2 (Rs; 34), and D11 (Gh; 2, 12). Each of the above mentioned proteins represents the family member or individual protein that is most similar

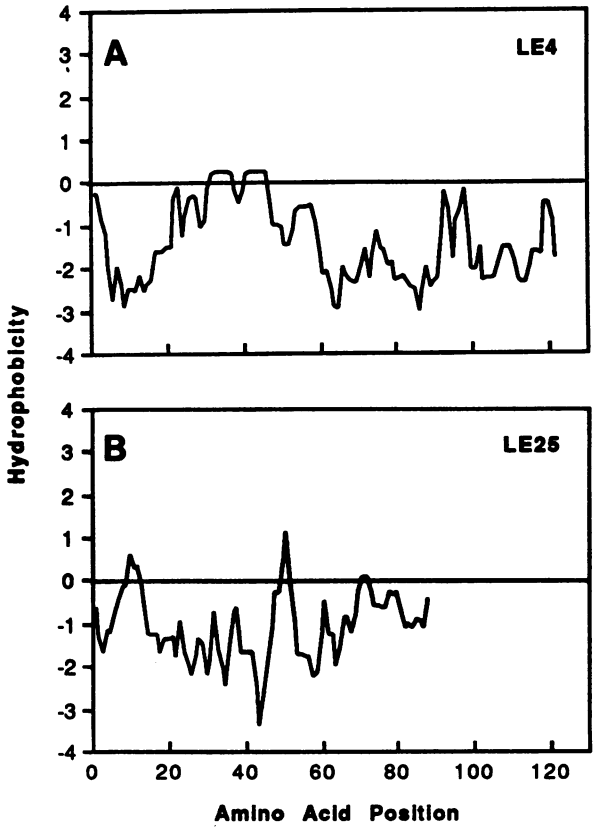

Figure 2. Hydropathy plots of the deduced polypeptides LE4 (A) and LE25 (B). Hydropathy plots were predicted according to the method of Kyte and Doolittle (24) using a window of seven amino acids. The abscissa represents the position of the amino acid residues. Negative values indicate hydrophilicity.

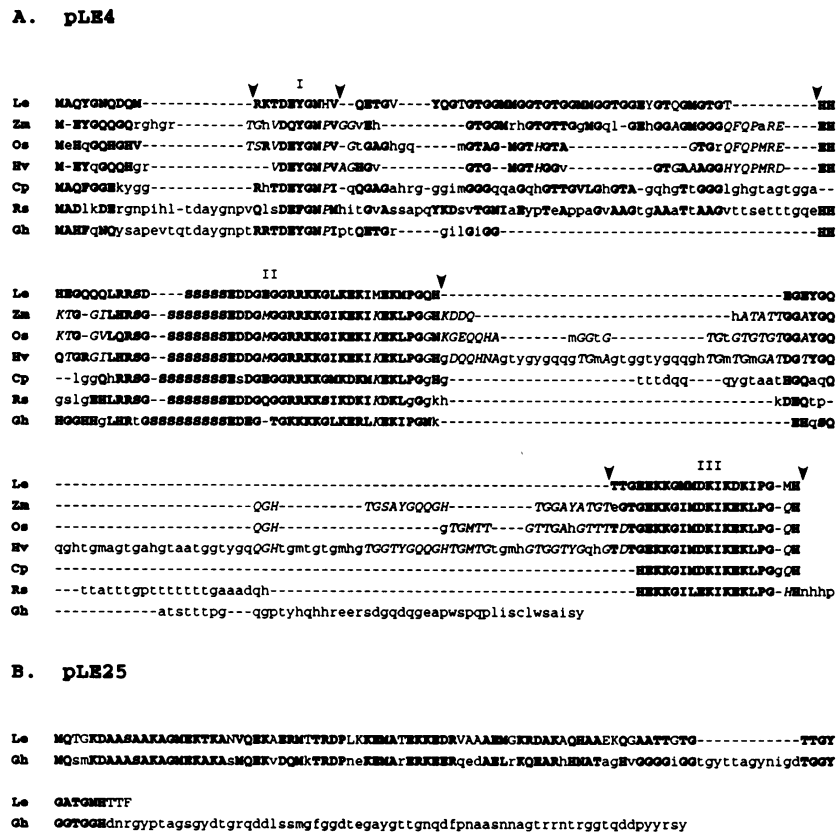

Figure 3. Sequence comparison of the deduced LE4 and LE25 polypeptides with previously described polypeptides. A, The LE4 (Le, *L. esculentum*) amino acid sequence is aligned with the predicted amino acid sequences of RAB-17 (Zm, *Zea mays*; 40), RAB 16A (Os, *Oryza sativa*; 41), DHN4 (Hv, *Hordeum vulgare*; 8, 9), pcC6–29 (Cp, *C. plantagineum*; 30), RSLEA2 (Rs, *Raphanus sativa*; 34), and D11 (Gh, *Gossypium hirsutum*; 2, 12). Bold letters denote amino acid identity and similarity >0.4 to the LE4 polypeptide; italicized letters represent identity or similarity >0.4 between the grasses or where all polypeptides except LE4 are similar; and lower case letters denote no similarity. Similarities were determined using the Dayhoff table (37). Three conserved motifs are numbered I, II, and III and marked with arrows to indicate the boundaries of the calculated identities and similarities. B, Alignment of the LE25 (Le) deduced amino acid sequence with that of D113 (Gh; 2). Amino acid identity and similarity were compared as described above.

to LE4 with 38 to 60% identity at the amino acid level. Alignment of the amino acid sequences shows that there are three conserved regions (I, II, and III; Fig. 3A). Most highly conserved among all seven polypeptides is region II, rich in the amino acid residues Ser and Lys. The polypeptides have a 58 to 70% identity to LE4 within this region. Region III at the carboxy terminus, also rich in the amino acid Lys, is highly conserved among all of the polypeptides except D11. Region I contains the amino acid sequence Asp-Glu-Tyr-Gly-Asn (DEYGN; Fig. 3A) conserved among five of the seven polypeptides. The Zm and Rs polypeptides contain one amino acid substitution within this sequence.

The pLE25 deduced amino acid sequence is similar to the cotton late embryogenesis abundant protein D113 (2). A sequence comparison at the amino acid level is shown in Figure 3B. The LE25 polypeptide (Le) is 77 amino acids shorter than D113 (Gh). There is a 64% similarity and 50% identity between the two polypeptides. These percentages increase to 90% similarity and 75% identity when the first 20 amino acids are compared. Both LE25 and D113 are rich in the amino acids Ala and Gly, while lacking Cys and Trp.

pLE4 and pLE25 mRNA Accumulation in Different Organs

The pLE4 and pLE25 mRNAs accumulated in petioles and stems of detached, drought-stressed shoots and not in the corresponding nonstressed organs of 2- to 3-month-old plants (Fig. 4A). Similarly, pLE4 mRNA accumulated to high levels in detached, drought-stressed leaves (Fig. 4B). In contrast, this mRNA was at the limit of detection in seeds of B, T, P, and R fruit and was not detected in seeds harvested from fruit at the MG stages of development (Fig. 4B). No accumulation of this transcript was detected in the pericarp of MG or R fruit (Fig. 4A). pLE25 mRNA accumulated to very high levels in developing seeds compared with detached, drought-stressed leaves (Fig. 4B). High levels of the transcript were first detected in the seeds of MG2 fruit and remained at approximately the same level in seeds of MG3 and 4 fruit. An increase in accumulation occurred in the seeds of B fruit, before a gradual decrease in seeds of T, P, and R fruit. pLE25 mRNA also accumulated at low levels in the pericarp of R fruit (Fig. 4A). It should be noted that the RNA blots in Figure 4A and B were exposed to x-ray film for different lengths of time. Therefore, the levels of pLE4 and pLE25 mRNA accumulation cannot be compared directly between the two sets of blots.

ABA Accumulation during Tomato Seed Development

ABA levels increased and decreased twice during the eight stages of seed development that were investigated in tomato (Fig. 5A). Seeds of MG1 fruit had an ABA level of 2200 ng/g fresh weight. This level increased 2.5-fold in seeds of MG2 fruit. From the MG2 to 4 stage of fruit development, seed ABA levels decreased approximately 2.5-fold to the level measured in seeds of MG1 fruit. A second rise in seed ABA to 4100 ng/g fresh weight occurred at the B stage of development. This increase was followed by a twofold decrease in seeds of T fruit and further decreases in seeds of P and R fruit.

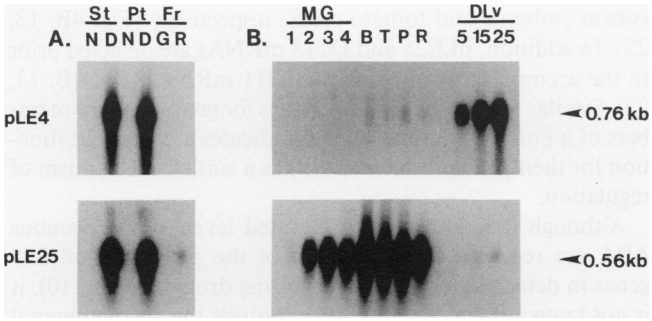

Figure 4. Accumulation of pLE4 and pLE25 mRNAs in different plant tissues. A, Total RNA was isolated from detached, drought-stressed (D), and nonstressed (N) petioles (Pt) and stems (St) and fruit (Fr) at the mature green (G) and red (R) stages. Thirty micrograms of RNA was size-separated in a formaldehyde 1.2% agarose gel, transferred to Zeta-Probe membrane, and hybridized with a ^{32}P-labeled antisense RNA probe synthesized from either the pLE4 or pLE25 cDNA insert. B, Total RNA was isolated from seeds of tomato fruit at eight developmental stages (MG1, 1; MG2, 2; MG3, 3; MG4, 4; B; T; P; R) and detached, drought-stressed leaves (DLv). Twenty-five micrograms of seed RNA and 5, 15, and 25 μg of drought-stressed leaf RNA were analyzed as described above.

Fresh and Dry Weights of Tomato Seeds during Mature Fruit Development

Seeds of tomato cv Ailsa Craig reached a peak fresh weight at the MG2 stage of fruit development and decreased in fresh weight thereafter (Fig. 5B). A 50% decrease in seed fresh weight occurred between the MG2 and 4 stage of fruit development, followed by a second decrease between the B and P stage. At the R stage of fruit development, seed fresh weight increased slightly to the weight measured in seeds of T fruit. With respect to seed dry weight, there was a small and gradual increase between the MG1 and T stages of fruit development (Fig. 5B). This increase in seed dry weight was followed by a slight decrease in seeds of P fruit and no change between the P and R stages of fruit development.

Accumulation of pLE4 and pLE25 mRNAs in Response to Different Abiotic Stresses

Three-week-old, hydroponically grown plants were subjected to several different abiotic stresses. The pLE4 and pLE25 mRNAs accumulated primarily in the leaves of drought-stressed plants and the roots of NaCl- and ABA-treated plants (Fig. 6). Accumulation of pLE4 mRNA was also detected at low levels in the leaves and roots of PEG-treated plants, the leaves of nonstressed and NaCl-treated plants, and the roots of plants given a cold treatment. pLE25 mRNA was detected at low levels in the roots of drought-stressed and PEG-treated plants. The pLE25 and pLE4 RNA blots were exposed to x-ray film for the same length of time.

ABA Levels in the Leaves and Roots of Plants Exposed to Different Abiotic Stresses

Each stress treatment, with the exception of heat stress, caused an increase in the leaf endogenous ABA level that was greater than the level measured in leaves of nonstressed plants (Fig. 7). The ABA levels in the leaves of drought-stressed and PEG-treated plants were the highest, followed by the level measured in NaCl- and ABA-treated plants, and last cold-treated plants. Root ABA levels were lower than leaf ABA levels, with the exception of ABA-treated plants with a bulk ABA level just over 8200 ng/g fresh weight (Fig. 7). The ABA levels in the roots of NaCl-, cold-, and heat-stressed plants were below the level measured in the roots of nonstressed plants. The roots of PEG-treated plants had an ABA level just slightly above the level measured in roots of nonstressed plants, whereas the roots of drought-stressed plants had a level of ABA that was 3.4-fold higher than the level measured in roots of nonstressed plants (Fig. 7).

DISCUSSION

Three major homology groups of ABA-responsive genes have been distinguished by sequence identities at the nucleo-

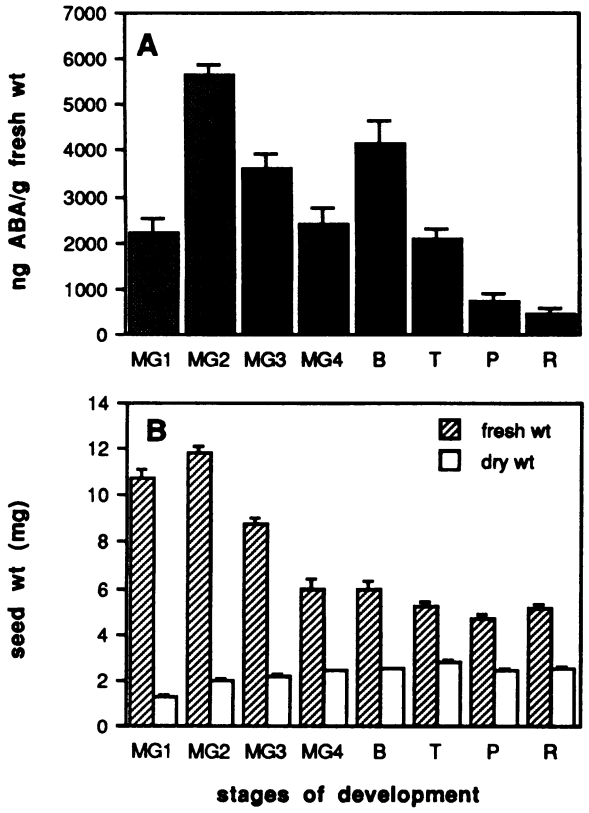

Figure 5. A, ABA concentration in developing tomato seeds. ABA was extracted from seeds of fruit at eight developmental stages (MG1, MG2, MG3, MG4, B, T, P, R) and quantified using an ABA radioimmunoassay. Data represents the average ±SE of five to eight replications on individual samples of 10 to 20 seeds. B, Fresh and dry weight of developing tomato seeds. The fresh (hatched bar) and dry (open bar) weights were determined for seeds taken from fruit at eight developmental stages (MG1, MG2, MG3, MG4, B, T, P, R). Seeds were removed from the fruit and weighed to determine fresh weight. After oven-drying, seed dry weight was determined. Data represents the average ± SE of 20 to 30 replications of individual seeds.

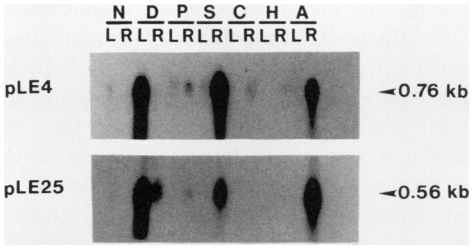

Figure 6. Accumulation of pLE4 and pLE25 mRNAs in leaves and roots of hydroponically grown tomato plants exposed to abiotic stress treatments. Total RNA was isolated from the leaves (L) and roots (R) of nonstressed (N), drought-stressed (D), PEG-treated (P), NaCl-treated (S), cold-treated (C), heat-stressed (H), and ABA-treated (A) plants. Thirty micrograms of RNA was analyzed as described in the legend of Figure 4.

tide and amino acid levels (12, 38). The cDNA pLE4 represents an ABA-induced gene, present in the tomato genome as a single copy (6), that is homologous to the *dhn* family in barley and maize (8, 9), the *rab* family in maize (40) and rice (41), pcC6-19 and pcC27-04 in *C. plantagineum* (30), and the cotton D11 (2, 12) and radish RSLEA2 (34) genes. The genes within this homology group are expressed in wilted vegetative tissues and/or developing seeds. The coding sequence of pLE4 is identical to the salt- and ABA-induced cDNA TAS14 also isolated from tomato (15); however, pLE4 contains an additional 62 nucleotides at the 5′ end and two additional nucleotides at the 3′ end before the poly(A) tail. The LE4 polypeptide contains three regions (I, II, and III) that are highly conserved among the members of its homology group (Fig. 3A). The conservation of these regions is greater among the members derived from monocotyledonous plants than between the mono- and dicotyledonous members of this group. These comparisons can be used to identify amino acid residues that do not diverge among all of the predicted polypeptides and, therefore, may be particularly important to a conserved structure or function among this group of proteins. For example, 12 out of 21 residues in region III and only 5 consecutive Ser residues in region II are conserved in the related polypeptides. It is not know at this time if these conserved domains, or any unique features of the deduced LE4 polypeptide, are significant to the structure or function of the LE4 protein.

The cDNA pLE25, which also represents a single-copy gene (6), encodes a deduced polypeptide that is similar to the cotton protein D113 (2) and, therefore, enables identification of an additional homology group. A comparison of the two polypeptides, including introduced gaps, showed that the shorter LE25 amino acid sequence aligns with the first 100 amino acids of D113 (Fig. 3B). If these two proteins have a similar function, the essential domains may have been retained within the LE25 amino acid sequence.

The temporal accumulation of pLE4 and pLE25 mRNA and the level of each transcript in developing tomato seeds were different from each other (Fig. 4B), yet similar to their homologs D11 and D113 whose mRNAs accumulate during cotton embryogenesis (13, 22). D113 and pLE25 mRNAs accumulate to a greater level than D11 and pLE4 mRNA in cotton embryos and tomato seeds, respectively (Fig. 4B; 13, 22). In addition, pLE25 and D113 mRNAs are detected prior to the accumulation of pLE4 and D11 mRNAs (Fig. 4B; 13, 22). Similar expression characteristics for genes that are members of a homology group further indicates a conserved function for their gene products as well as a similar mechanism of regulation.

Although it is known that elevated levels of endogenous ABA are required for expression of the pLE4 and pLE25 genes in detached tomato leaves during drought stress (10), it is not known if endogenous ABA controls the developmental expression of these genes. The endogenous ABA levels in developing seeds were elevated above those measured in drought-stressed leaves (Figs. 5A and 7). During the stages of seed development when ABA levels were elevated, pLE25 mRNA accumulated to high levels (Figs. 4B and 5A). D113 expression during the cotyledon and maturation stages of cotton embryogenesis is also associated with elevated ABA levels (22). These results indicate that ABA may be involved in the regulation of specific seed genes, but do not establish ABA as the endogenous regulator of either pLE25 or D113 gene expression. Unlike pLE25 mRNA accumulation, pLE4 mRNA did not accumulate to high levels in developing seeds and was only detected in the late stages of seed development when ABA levels were declining (Figs. 4B and 5A). RAB-17 mRNA, related to pLE4 mRNA, accumulates in drought-stressed maize leaves, mature embryos (31), and ABA-treated calli (40). RAB-17 mRNA accumulation is not detected in drought-stressed, ABAdeficient *vp2* mutant leaves of maize, but does accumulate to low levels in mutant embryos (31). These results indicate that the ABA-responsive gene RAB-17 may not be regulated by endogenous ABA in developing embryos. Future gene expression studies using the ABA-deficient tomato mutant, *flacca,* will help to further determine the role of ABA in the regulation of gene expression during seed development.

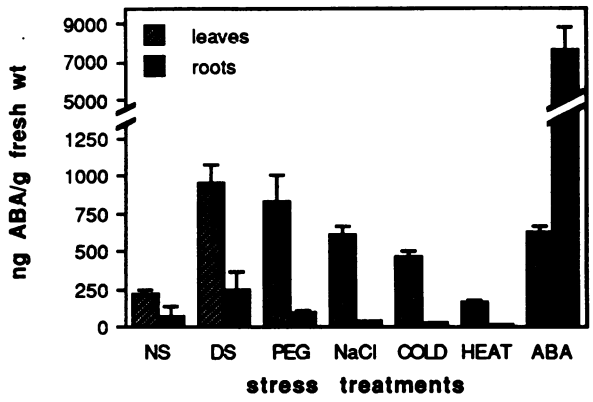

Figure 7. ABA content in leaves and roots of hydroponically grown tomato plants exposed to abiotic stress treatments. ABA was extracted from the leaves (hatched bar) and roots (solid bar) of nonstressed (NS), drought-stressed (DS), PEG-treated (PEG), NaCl-treated (NaCl), cold-treated (COLD), heat-stressed (HEAT), and ABA-treated (ABA) plants and quantified using an ABA radioimmunoassay. Data represents the average ± SE of three to five replications on individual tissue samples.

pLE4 and pLE25 mRNA accumulation occurred in response to specific abiotic stresses in an organ-specific manner. The patterns and levels of pLE4 and pLE25 mRNA accumulation were similar in response to specific abiotic stresses, unlike the accumulation of these two mRNAs in seeds (Figs. 4B and 6). The major difference in the accumulation patterns of the pLE4 and pLE25 transcripts was that pLE4 mRNA did not accumulate in detached, drought-stressed roots (Fig. 6). This contrasted with the expression of genes homologous to pLE4 in drought-stressed roots of wheat, barley, and corn (8, 9) and *C. plantagineum* (30). In addition, salt-treated tomato plants, grown until five to six true leaves were present, accumulated TAS14 mRNA in leaves and roots (15). In the experiments reported here, 3-week-old tomato plants grown and treated in a similar manner only accumulated pLE4 mRNA in roots (Fig. 6). Variations in the age of the plants studied may explain differences between the results reported here and those previously published. There was also evidence that pLE25 expression was altered by the age of the plant. In detached, drought-stressed leaves removed from 3-month-old plants, pLE4 mRNA accumulated to higher levels than the pLE25 transcript (Fig. 4B). However, an approximately equal accumulation of both transcripts was observed in detached, drought-stressed leaves of 3-week-old, hydroponically grown tomato plants (Fig. 6). These results point to a potential developmental component to the regulation of gene expression in response to stress. The similar expression characteristics of pLE4 and pLE25 in response to different stresses, yet dissimilar developmental expression, indicates that there are at least two mechanisms of regulation.

It is also not known if endogenous ABA regulates the accumulation of the pLE4 and pLE25 transcripts in different organs in response to different stresses. The ABA levels and accumulation of each transcript did correspond in leaves and roots of plants exposed to specific abiotic treatments (Figs. 6 and 7). The leaves and roots of nonstressed and heat-shocked plants did not accumulate high levels of ABA or either transcript. Both ABA and mRNA levels increased in the leaves of drought-stressed plants and the roots of PEG- and ABA-treated plants. These results, in addition to our previous observations (10), support ABA as a regulator of pLE4 and pLE25 gene expression in these organs under specific conditions. Other stresses, PEG, salt, cold, and ABA treatments, resulted in little or no accumulation of either transcript in leaves. ABA levels in the leaves of these treated plants were higher than those in nonstressed leaves, yet still below the level in detached, drought-stressed leaves (Fig. 7). It was previously shown that detached leaves treated with 100 μM ABA accumulate pLE4 and pLE25 mRNA, but those treated with a lower concentration of ABA (10 μM) do not (10). Therefore, a threshold level of ABA may be required for ABA-induced gene expression in leaves. Conversely, ABA levels were not elevated in the roots of drought-stressed or salt-stressed plants in which pLE25 mRNA, and pLE4 and pLE25 mRNA accumulated, respectively. Several potential explanations exist for the discrepancies between endogenous ABA levels and the accumulation of pLE4 and pLE25 mRNA. First, different abiotic stresses may alter the sensitivity of the pLE4 and pLE25 genes to ABA. Second, the level of endogenous ABA required for expression in leaves may be different from that of roots. Third, the bulk ABA level in stressed organs may not reflect the level of ABA at the active site. And fourth, regulation of pLE4 and pLE25 gene expression may not require elevated levels of ABA during all abiotic stresses, as it does during drought stress. Until ABA recognition at the cellular level is understood, it may not be possible to fully comprehend the regulatory mechanism of gene expression by ABA.

Although many of the results presented here further our understanding of ABA-regulated gene expression, they also point to the complexity of the regulation of gene expression by ABA. pLE4 and pLE25 represent ABA-induced genes that are differentially regulated during seed development, yet are regulated in a similar manner in response to specific abiotic stresses. Therefore, separate mechanisms may exist for environmental and developmental regulation of these genes. Future studies focusing on potential ABA-responsive DNA sequence elements within the pLE4 and pLE25 genes are needed to further our understanding of ABA-regulated gene expression.

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