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Characterization of three mRNAs that accumulate in wilted tomato leaves in response to elevated levels of endogenous abscisic acid

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Abstract. The accumulation of abscisic acid (ABA) has been shown to regulate some of the changes in gene expression which occur during water deficit. In order to characterize these ABA-induced changes, we have identified and isolated three copy DNAs (cDNAs) that represent genes which are expressed in response to ABA during drought stress. The ABA-deficient mutant of tomato, *flacca*, synthesizes low levels of ABA during water deficit compared to the wild type (WT) (*Lycopersicon esculentum* Mill. cv. Ailsa Craig). The mutant *flacca* was used to distinguish cDNAs corresponding to mRNAs which accumulate during water deficit in response to elevated levels of ABA from those mRNAs which are not ABA responsive. A cDNA library representing the mRNA population of wilted WT tomato leaves was constructed and a series of differential screens was used to select cDNAs that represent putative ABA- and drought-induced mRNAs. Three cDNAs were isolated from the screens and were identified as pLE4, pLE16, and pLE25. The corresponding mRNAs were preferentially expressed in wilted WT leaves and were not expressed in wilted ABA-deficient mutant leaves. The inability of the mutant to accumulate these drought-induced transcripts was reversed with exogenously applied ABA. A correlation was observed between the accumulation of the drought-induced mRNAs and the endogenous ABA levels measured in WT leaves throughout increasing periods of water deficit. These results indicate that endogenous ABA regulates the accumulation of pLE4, pLE16, and pLE25 mRNAs in tomato leaves during water deficit.

Key words: Abscisic acid and drought stress – Drought stress – Gene expression – *Lycopersicon* (ABA and gene expression) – Water deficit

Introduction

Water deficit results in an increase in the biosynthesis of the plant hormone abscisic acid (ABA) (Wright and Hiron 1969). The accumulation of ABA in leaves causes stomatal closure (Walton 1980), which limits the transpiration rate and aids the plant in its conservation of water. Changes in gene expression also occur in response to water deficit. For example, total protein synthesis is reduced during water deficit, while the synthesis of specific mRNAs and polypeptides is increased (Bewley et al. 1983; Dasgupta and Bewley 1984; Bray 1988). The accumulation of specific proteins in response to water deficit has been described in several plants including maize (Bewley et al. 1983), pea (Lalonde and Bewley 1986), barley (Jacobsen et al. 1986), tomato (Bray 1988), and rice (Mundy and Chua 1988). Drought-induced changes in the population of translatable mRNA also have been reported (Bewley and Larsen 1982; Bensen et al. 1988; Bray 1988; Guerrero and Mullet 1988).

While the mechanism(s) that control(s) drought-induced changes in gene expression is not fully understood, there is evidence to indicate that ABA coordinates plant responses to water deficit. Several researchers have demonstrated that exogenous ABA applications and water deficit both result in specific alterations in gene expression. Studies by Heikkila et al. (1984) showed that several proteins are synthesized de novo in response to water deficit and ABA treatments. In-vitro translation products have been used to demonstrate that changes in the mRNA population occur during drought stress or in response to ABA applications (Bray 1988; Guerrero and Mullet 1988). Specific mRNAs that accumulate during the desiccation phase of seed embryogenesis are also modulated by ABA treatments (Galau et al. 1986; Gomez et al. 1988; Mundy and Chua 1988; Harada et al. 1989). These studies, involving exogenous ABA treatments, indicate that the expression of specific genes is regulated by ABA, but they cannot confirm that endogenous ABA plays a regulatory role in the alteration of gene expression in response to water deficit.

The ABA-deficient mutant of tomato, *flacca*, which contains a block in its ABA biosynthetic pathway (Parry et al. 1988), has a reduced level of ABA compared to the wild-type (WT) Ailsa Craig (Neill and Horgan 1985). In well-watered plants, the ABA level in *flacca* leaves is approx. 50% of the level in Ailsa Craig leaves (Bray 1988). In response to a 6-h water deficit, the ABA level in the mutant is only 6% of that found in the WT. This significant difference in the ABA levels measured

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Abbreviations: ABA = abscisic acid; cDNA = copy DNA; poly(A)$^+$ = polyadenylated; WT = wild-type

in the stressed genotypes results from a 22-fold increase in ABA in wilted WT leaves, and only a threefold increase in wilted mutant leaves (Bray 1988). Because of its reduced ABA content and impaired stomatal regulation, the mutant has a wilty phenotype (Tal 1966; Bradford 1987). However, treatment of the mutant with ABA reverts the wilty phenotype to normal (Tal and Nevo 1973).

A comparison of protein and mRNA accumulation patterns in *flacca* and WT leaves in response to water deficit and ABA applications has demonstrated that changes in endogenous ABA levels are responsible for some drought-induced alterations in gene expression (Bray 1988). *Flacca* does not synthesize drought-specific polypeptides in response to water deficit (Bray 1988). This set of polypeptides is synthesized in mutant leaves only after exogenous ABA treatment. Similarly, a set of in-vitro translation products is synthesized from mRNAs isolated from drought-stressed WT leaves that is not synthesized from mRNAs isolated from drought-stressed mutant leaves. In-vitro translation of mRNAs isolated from ABA-treated mutant leaves demonstrated the presence of translation products similar to those synthesized from mRNAs of drought-stressed WT leaves (Bray 1988).

In this study, the ABA-deficient mutant *flacca* and its WT Ailsa Craig were used to investigate the regulation, by endogenous ABA, of specific changes in gene expression that occur in response to water deficit. A comparison between the hybridization of wilted WT and mutant leaf mRNA populations, to a copy-DNA (cDNA) library constructed from polyadenylated (poly(A)$^+$)RNA isolated from wilted WT leaves, was used to isolate three cDNAs: pLE4, pLE16, and pLE25. The mRNAs corresponding to these cDNAs were drought-induced and required elevated levels of endogenous ABA for their expression in tomato leaves. The accumulation of these mRNAs over time was found to correlate with endogenous ABA levels measured in wilted WT leaves.

Material and methods

Plant material. Lycopersicon esculentum Mill. cv. Ailsa Craig and the near isogenic, ABA-deficient mutant *flacca* (obtained from Dr. J.W. Maxon Smith, Glasshouse Crops Research Institute, Littlehampton, West Sussex, UK) were grown in a greenhouse for two to three months.

Experimental treatments. All treatments were completed on detached, expanding tomato leaves at 28° C under 102.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light. Leaves were removed from the plant and immediately placed in water. Leaves were wilted to 88% of their original fresh weight on the laboratory bench, and then maintained in their wilted state in clear plastic bags (10 cm wide, 15 cm long) for the duration of the stress. For time-course studies, leaves were wilted and then placed in plastic bags for periods of 30 min to 24 h. Nonstressed leaves were placed in water for the treatment period. All ABA treatments were applied to leaves by placing the petioles in a solution of 10^{-3}, 10^{-4}, or 10^{-5} M (±)-ABA (Sigma Chemical Co., St. Louis, Mo., USA) for 6 h. Leaflets were frozen in liquid nitrogen and stored at −80° C.

RNA isolation. Total RNA was extracted from tomato leaflets (Prescott and Martin 1987), and poly(A)$^+$RNA was isolated by oligo-dT cellulose (type 7; Pharmacia, Piscataway, N.J., USA) affinity chromatography as described by Maniatis et al. (1982).

Construction and screening of a cDNA library. Polyadenylated RNA, isolated from WT tomato leaves that were wilted for 6 h, was used to construct a cDNA library in the phage vector lambda GEM-4 (Promega, Madison, Wis., USA) as described by Huynh et al. (1985) with the following modifications. An oligo-dT XbaI primer-adapter was used (Promega) to prime first-strand cDNA synthesis. EcoRI/XbaI-digested cDNA was ligated into EcoRI/XbaI-digested lambda GEM-4. The library was plated on the bacterial host strain LE392 at 10000 plaque-forming units (pfu)/plate (150 mm diameter). The cDNA library was screened using a differential plaque hybridization method (St. John and Davis 1979). Two sets of nitrocellulose (0.45 µm; Micro Separations, Westboro, Mass., USA) plaque replicas were prepared according to Maniatis et al. (1982) and hybridized with ^{32}P-labeled reverse transcripts of mRNA isolated from wilted WT or *flacca* leaves. Random-primed cDNA was synthesized in a 30-µl reaction mixture containing 3 µg of poly(A)$^+$RNA, 12.5 units RNAsin (Promega), 50 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride (Tris-HCl; pH 8.0), 8 mM $MgCl_2$, 40 mM KCl, 200 µM each of dATP, dGTP, and dTTP, 5 µM dCTP, 4 mM dithiothreitol, 0.02% Nonidet P-40 (Sigma Chemical Co.), 300 µg/ml of the random hexamer oligonucleotide primer pd(N)$_6$ (Pharmacia), 2.2 mBq α-[^{32}P]dCTP (111 TBq/mmol; Amersham, Arlington Heights, Ill., USA), and 30 units of AMV reverse transcriptase (Siekagaku America, St. Petersburg, Fla., USA). Following a 3-h incubation at 37° C, the reactions were stopped by the addition of 2 µl of 0.5 M ethylenediaminetetraacetic acid (EDTA; pH 8.0). Following the addition of salmon sperm DNA (50 µg), the reactions were extracted with phenol:chloroform (1:1, v/v), applied to a G-50 Sephadex (fine-DNA grade; Pharmacia) column, butanol-extracted, and ethanol-precipitated. Replica filters were hybridized with $1.3 \cdot 10^7$ cpm/ml of the WT or mutant probe at 42° C and washed according to Wahl and Berger (1987). The hybridization buffer included 100 µg/ml polyuridylic acid. The final wash was in 0.1 × SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.5), 0.1% sodium dodecyl sulfate (SDS) for 30 min at 65° C. Plaques which hybridized only to the WT probe and not to the mutant probe were selected, plated at a density of 100–200 pfu/plate (100 mm diameter), and rescreened using the method described above.

Subcloning and cross hybridizations. DNA, prepared from each of the selected plaques according to Maniatis et al. (1982), was digested with the restriction enzyme SpeI, and ligated to release the pGEM-1 plasmid containing a cDNA insert (Palazzolo and Meyerowitz 1987). Cross-hybridizations were performed to determine which of the plasmids contained unique cDNA inserts. Linearized plasmid DNAs and pGEM-1 were blotted onto a nitrocellulose membrane (0.45 µm; Schleicher and Schuell, Keene, N.H., USA) using a slot blot manifold (Schleicher and Schuell). High-specific activity RNA probes were synthesized using T7 RNA polymerase (Promega Riboprobe System Technical Bulletin, modified from Melton et al. 1984). The blots were hybridized with $1 \cdot 10^6$ cpm/ml of an RNA probe and washed according to Promega (Notes, March 1985).

RNA blot analyses. Thirty micrograms of total RNA was separated by size in a formaldehyde denaturing 1.2% agarose gel according to Maniatis et al. (1982). The RNA was transferred to Hybond-N membranes (0.45 µm; Amersham) using 20 × SSC as a transfer medium. The RNA was cross-linked to the membranes by exposure to transmitted ultraviolet (UV) irradiation of 300 nm for 3 min. The membranes were prewashed in a solution of 5 mM Tris-HCl (pH 8.0), 2 mM EDTA (pH 8.0), and 0.1 × Denhardt's solution (0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone) at 65° C for 2 h to minimize nonspecific hybridization. Membranes were prehybridized for 4 h at 42° C in 50% formamide, 5 × SSPE (1 × SSPE = 0.18 M NaCl, 0.01 M sodium phosphate, 8 mM NaOH, 1 mM EDTA, pH 7.5), 5 × Denhardt's solution,

0.6 mg/ml denatured salmon sperm DNA, 0.25 mg/ml polyadenylic acid (polyA), and 0.25 mg/ml *Escherichia coli* tRNA. Hybridization was continued overnight at 55° C in a solution of 50% formamide, 5 × SSPE, 2 × Denhardt's, 0.3 mg/ml denatured salmon sperm DNA, 0.05 mg/ml polyA, 0.05 mg/ml *E. coli* tRNA, and $1 \cdot 10^7$ cpm/ml of a random-primed, ^{32}P-oligo-labeled cDNA insert (Feinberg and Vogelstein 1983). Membranes were washed according to the manufacturer (Amersham), except that each wash time was doubled, and the final wash was repeated.

Two methods were used to ensure that the hybridization patterns obtained with all RNA blots were accurate. Each nylon membrane was stripped of its radioactive signal according to the manufacturer (Amersham) and rehybridized with a different cDNA insert. Membranes were also stained for ribosomal RNAs (Maniatis et al. 1982) to confirm that each blotted sample contained an equal quantity of RNA.

RNA slot hybridizations. A quantity of 1.25, 2.5, and 5.0 µg of total RNA from each sample was brought to a volume of 25 µl with diethyl pyrocarbonate-treated water (Maniatis et al. 1982) and was treated as described by Galau et al. (1987). The RNA was applied to Hybond-N (Amersham) using a slot blot manifold. DNA standards were used to quantify the level of a given pLE transcript. Each of the pLE plasmids containing a cDNA insert was linearized and dilutions were made to obtain a standard curve of 0.02–10 ng of insert DNA. The volume of each sample was adjusted to 100 µl and the DNA was denatured with 0.1 volumes of 3 M NaOH for 1 h at 65° C. Prior to slotting, each DNA sample was neutralized with two volumes of 1 M sodium-phosphate buffer (pH 6.9). Hybridizations and post-hybridization washes of the membranes were carried out as described for the RNA blot analyses with the following modifications. The prehybridization of the membranes was done at 50° C and all membranes were hybridized with $2.5 \cdot 10^6$ cpm/ml of an antisense RNA probe having a specific activity of $5 \cdot 10^8$ cpm/µg. The intensity of hybridization was measured by densitometry.

Quantitation of ABA. Abscisic acid was quantified using a competitive ABA-radioimmunoassay as described in detail by Bray and Beachy (1985).

Results

Isolation of cDNAs representing drought- and ABA-induced mRNAs. A cDNA library was constructed in lambda GEM-4 using poly(A)$^+$RNA isolated from wilted WT tomato leaves. For the initial screen, approximately one-fifth of the library, containing $4.7 \cdot 10^5$ recombinants/µg of poly(A)$^+$RNA, was plated. Putative cloned drought- and ABA-induced mRNAs were identified using the differential plaque hybridization method of St. John and Davis (1979). Duplicate nitrocellulose plaque lifts were made from each plate and hybridized with ^{32}P-labeled cDNA synthesized from mRNA isolated from leaves of wilted WT or wilted ABA-deficient mutant. Plaques which hybridized only to the WT probe, and not to the mutant probe, contained copies of putative drought- and ABA-induced mRNAs and were selected for further characterization. Fifty-one plaques were selected from the initial screen of the library and were individually replated at a density of 100–200 plaque-forming units/plate. Duplicate plaque lifts of each plate were rescreened as described above. In the secondary screen, approximately half of the originally selected plaques hybridized exclusively with radiolabeled cDNA synthesized from leaf mRNAs isolated from wilted WT. Cross hybridization of recombinant plasmid subclones demonstrated that nine unique clones had been isolated. Each contained a cDNA insert greater than 500 basepairs (Table 1).

Table 1. Size comparison of the isolated cDNAs and their corresponding drought- and ABA-inducible mRNAs

	cDNA (kilobase pairs)	mRNA (kilobases)
pLE4	0.75	0.76
pLE16	0.70	0.70
pLE25	0.55	0.56
pLE8[a]	0.80	0.80

[a] The cDNA, pLE8, represents an mRNA which accumulates in nonstressed WT and mutant leaves and whose accumulation is not affected by ABA

Identification of drought-induced mRNAs. In order to determine if the nine cDNAs represented mRNAs which accumulated in response to water deficit, RNA gel blot analyses were performed. Total RNA was isolated from wilted and well-watered WT and ABA-deficient mutant leaves. RNA was size-separated using formaldehyde-agarose gel electrophoresis and blotted onto nylon membranes. Each RNA gel blot was hybridized with a random-primed, oligo-labeled insert of one of the nine unique cDNAs. Three of the cDNAs, pLE4, pLE16, and pLE25, represented mRNAs which accumulated preferentially in wilted WT leaves and not in wilted mutant

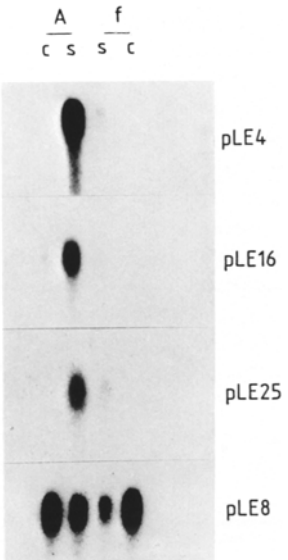

Fig. 1. Drought-induced accumulation of pLE4, pLE16, pLE25, and pLE8 mRNAs in leaves of WT (Ailsa Craig) tomato and an ABA-deficient mutant (*flacca*). Total RNA was isolated from detached Ailsa Craig (*A*) and *flacca* (*f*) leaves which had been drought-stressed (*S*) or maintained in water (*C*) for 6 h. Thirty micrograms of RNA were size-separated in a formaldehyde-1.2% agarose gel, transferred to Hybond-N membrane, and hybridized with a ^{32}P-labeled random-primed cDNA insert of pLE4, pLE16, pLE25, or pLE8

or nonstressed WT and mutant leaves (Fig. 1). While each of these mRNAs preferentially accumulated in leaves which produce high levels of endogenous ABA in response to water deficit (Bray 1988), pLE4 and pLE25 mRNAs were detected at very low levels in wilted *flacca* leaves and pLE16 was detected in nonstressed WT leaves. Detection of the pLE4 and pLE25 transcripts in stressed *flacca* leaves may have resulted from the mutant's ability to synthesize some additional ABA when drought-stressed (Bray 1988), despite a block in it's ABA biosynthetic pathway. Similarly, drought-induced polypeptides and in-vitro translation products were detected at low levels in wilted *flacca* leaves compared to wilted WT leaves (Bray 1988). Detection of pLE16 mRNA in nonstressed WT leaves demonstrated that drought-induced genes may also be expressed at low levels under nonstressed conditions.

The pLE8 clone was included in this study because it represented an mRNA species present in both WT and ABA-deficient mutant leaves under nonstressed conditions (Fig. 1). In response to water deficit, the level of the pLE8 mRNA decreased slightly in both WT and mutant leaves compared to the level detected in nonstressed leaves. The pLE8 clone was originally selected from the cDNA library as a plaque which showed greater hybridization to a cDNA probe synthesized from wilted WT leaf mRNA than a comparable mutant probe. Although pLE8 mRNA is not regulated by drought stress like the other mRNAs, it was isolated by this method because the drought-induced decrease in the level of this transcript was greater in the mutant than in the WT at 6 h.

The sizes of the mRNAs were all smaller than one kilobase. Determination of the transcript sizes showed that each of the cDNA inserts was approximately equal in size to the mRNA it represented (Table 1).

Induction of mRNAs in response to ABA. The drought-induced mRNAs did not accumulate in the leaves of the ABA-deficient mutant, indicating that elevated endogenous ABA levels were required for their unique accumulation. Abscisic acid treatments were used to determine if the accumulation of the mRNAs was induced by ABA. Detached WT and ABA-deficient mutant leaves were treated with water, 10^{-3}, 10^{-4}, or 10^{-5} M ABA for 6 h. RNA was extracted from these leaves, separated by size, blotted to a membrane, and hybridized with a ^{32}P-labeled insert of either pLE4, pLE16, pLE25, or pLE8. The drought-induced mRNAs accumulated in ABA-treated WT and mutant leaves to approximately the same level observed in WT leaves during water deficit (Fig. 2). Maximal levels of the mRNAs were observed in leaves treated with 10^{-4} M ABA. Either a lower or a higher concentration of ABA resulted in reduced accumulation of these mRNAs. However, the accumulation of pLE25 mRNA in WT leaves in response to the 10^{-3} M ABA treatment did not show a decrease with respect to the 10^{-4} M ABA treatment. The only transcript which was detected in response to a 10^{-5} M ABA treatment in WT leaves was pLE16. Therefore, while exogenously applied ABA resulted in the accumulation

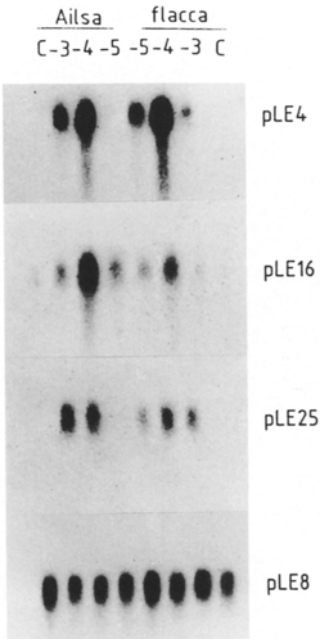

Fig. 2. Abscisic acid induced accumulation of pLE4, pLE16, pLE25, and pLE8 mRNAs in leaves of WT (*Ailsa*) tomato and an ABA-deficient mutant (*flacca*). Detached Ailsa-Craig and *flacca* leaves were treated with water (*C*), 10^{-3} (*−3*), 10^{-4} (*−4*) or 10^{-5} (*−5*) M ABA for 6 h. Total RNA was isolated from the treated leaves and 30 μg of RNA were analyzed as described in the legend of Fig. 1

of these drought-induced mRNAs in nonstressed WT leaves, differences were observed in the levels of mRNA which accumulated in response to a given ABA treatment. In mutant leaves, pLE16 and pLE25 mRNA levels were below the levels detected in WT leaves given the same ABA treatment. However, unlike WT leaves, mutant leaves accumulated pLE4 and pLE25 mRNAs in response to the lowest ABA concentration. The level of the pLE8 transcript, not induced by drought stress, was not altered by exogenously applied ABA in neither the mutant nor the WT (Fig. 2).

Accumulation of drought-induced mRNAs with increasing duration of stress. To determine the initial timing of expression, the pattern of accumulation, and the amount of each mRNA attained for a given time period of water deficit, total RNA was isolated from WT leaves which had been wilted for 30 min to 24 h. Hybridization of slot blots with radiolabeled RNA probes synthesized from the cDNA inserts of pLE4, pLE16, or pLE25 showed that each of the cDNAs represented an mRNA which rapidly accumulated in response to water deficit (Fig. 3B). The pLE4 transcript was detected within 1 h of water deficit, while pLE16 and pLE25 mRNAs were detected within 2 h of stress. The level of pLE16 and pLE25 mRNAs increased throughout the 24-h period of water deficit unlike the level of pLE4 mRNA which increased up to 16 h and then decreased. During the 24-h period of water deficit, the pLE16 transcript accumulated to the highest level (Fig. 3B). However, the pLE4 transcript accumulated to a greater level more rap-

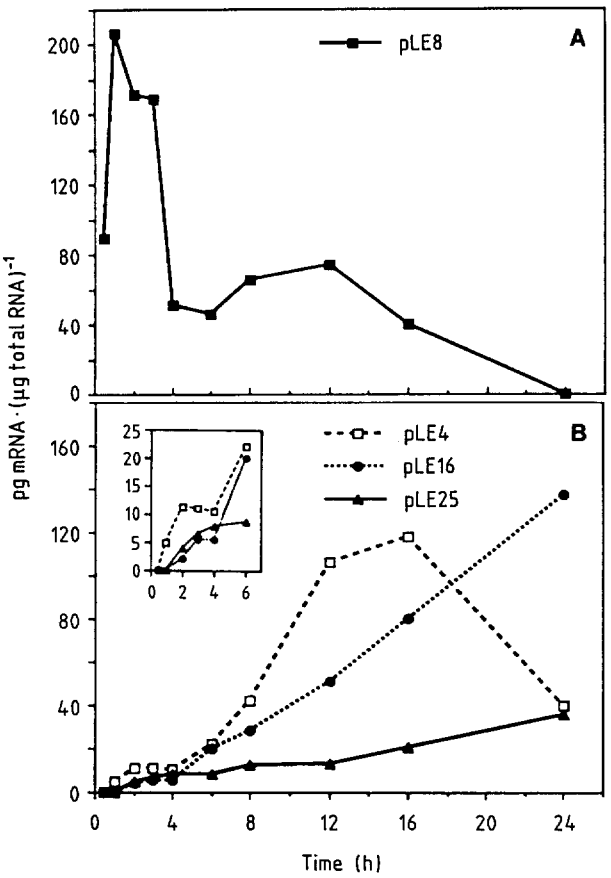

Fig. 3A, B. Quantitative analysis of the time courses of drought-induced accumulation of **A** pLE8 and **B** pLE4, pLE16, and pLE25 mRNAs in WT tomato (Ailsa Craig) leaves. Total RNA was isolated from Ailsa Craig leaves drought-stressed for the indicated amount of time (0.5, 1, 2, 3, 4, 6, 8, 12, 16 and 24 h), and RNA was slotted onto Hybond-N membrane and hybridized with a ^{32}P-labeled RNA probe synthesized from a pLE8 (■), pLE4 (□), pLE16 (●) or pLE25 (▲) cDNA insert. The level of a given transcript was determined by densitometry. Data represent the average of two replications. The *inset* shows the drought-stress period of 0–6 h in greater detail

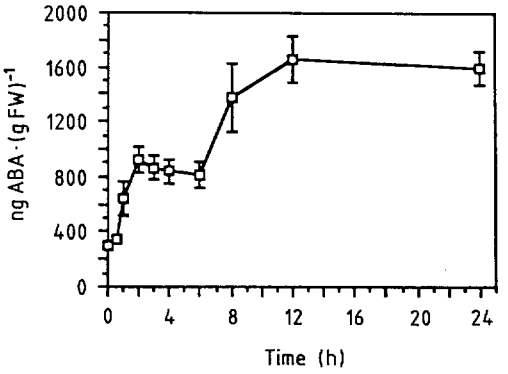

Fig. 4. Contents of ABA in detached drought-stressed WT (Ailsa Craig) tomato leaves. The leaves were drought-stressed for 30 min to 24 h; ABA was extracted from the leaves and quantified using an ABA-radioimmunoassay. Data represent the means and SE of four replications

idly than pLE16. The level of pLE25 mRNA was the lowest of the three transcripts. Beginning with the time point when the mRNAs were first detected, there was a ninefold increase in the level of pLE25 mRNA up to 24 h, a 27-fold increase in the level of pLE4 mRNA up to 16 h, and a 68.5-fold increase in the level of pLE16 mRNA up to 24 h. Unlike the three drought- and ABA-induced mRNAs, pLE8 mRNA levels increased twofold from 30 min to 1 h and then decreased with increasing periods of water deficit (Fig. 3A).

Endogenous ABA levels in wilted WT leaves. The level of ABA was determined at different time points over a 24-h period of water deficit using the radioimmunoassay described by Bray and Beachy (1985). An increase in ABA was measured as early as 30 min after the imposed water deficit (Fig. 4). This slight increase in ABA was followed by a twofold increase at 1 h and a threefold increase at 2 h over the ABA level measured in non-stressed WT leaves. Between 2 and 6 h of stress, ABA levels remained constant, and then sharply increased between 6 and 12 h. The level of ABA was 4.5-fold higher at 8 h and 5.5-fold higher at 12 h compared to the level measured in nonstressed WT leaves. Between 12 and 24 h of water deficit, no significant changes in the ABA levels were measured.

A correlation can be drawn between the pattern of endogenous ABA accumulation and that of pLE4, pLE16, and pLE25 mRNAs in wilted WT leaves over time. A rapid increase in both ABA and mRNA levels occurred within the first 2 h of water deficit (Figs. 3B, 4). The second increase in ABA, measured between 6 and 12 h of water deficit, was paralleled by an additional increase in the levels of pLE4 and pLE16 mRNAs. In the final 12 h of stress, the ABA levels remained the same; however, pLE16 and pLE25 mRNAs continued to accumulate during this period, while the level of pLE4 mRNA increased until 16 h before declining at 24 h.

Discussion

Copy DNAs representing mRNAs which preferentially accumulate in response to water deficit have previously been reported (Gomez et al. 1988; Guerrero and Mullet 1988; Mundy and Chua 1988). Several groups have identified genes expressed in seeds during the desiccation phase of embryogenesis that are also induced by exogenous ABA (Galau et al. 1986; Gomez et al. 1988; Mundy and Chua 1988; Close et al. 1989; Harada et al. 1989). However, only Guerrero and Mullet (1988) have isolated cDNAs representing drought-induced mRNAs from a cDNA library constructed from mRNAs isolated from the vegetative portion of the plant. Four cDNAs (C, D, E, and F) have been isolated which hybridize to mRNAs that accumulate in wilted pea shoots and not well-watered shoots. The mRNA hybridization patterns obtained with these pea-shoot cDNAs are similar to those observed with pLE4, pLE16, and pLE25 in wilted and well-watered WT tomato leaves. However, while Guerrero and Mullet (1988) have shown that the

ABA level in pea shoots rises 50-fold after 4 h of wilting, they have not demonstrated that the four wilted-shoot cDNAs represent mRNAs which accumulate in response to ABA.

Elevated levels of endogenous ABA are required for the regulation of the three mRNAs represented by the cDNAs pLE4, pLE16, and pLE25. These mRNAs accumulated preferentially in wilted WT leaves (Fig. 1), although treatment of well-watered WT and mutant leaves with exogenous ABA resulted in the accumulation of these transcripts (Fig. 2). The ability of the ABA-deficient mutant to accumulate these drought-induced mRNAs when ABA was exogenously applied demonstrated that these mRNAs are transcribed from genes present in both WT and mutant leaves. However, the mutant is unable to regulate the expression of these genes in response to water deficit because of its inability to accumulate ABA.

Drought-induced accumulation of pLE4, pLE16, and pLE25 mRNAs was rapid and found to correlate with changes in the endogenous ABA levels measured during increasing periods of water deficit in WT leaves (Figs. 3B, 4). Gomez et al. (1988) also observed that the level of ABA in wilted corn leaves correlated with accumulation of pMAH9 mRNA. Using developing cotton embryos, Galau et al. (1987) observed that maximal endogenous ABA levels occurred during maximal expression of early Class I *Lea* (late embryogenesis-abundant) mRNAs. During the later stages of embryogenesis, ABA levels decreased while Class I and II *Lea* mRNAs continued to accumulate. While we observed no decrease in the ABA levels in wilted leaves during the 24-h stress period, pLE16 and pLE25 mRNA levels continued to increase after ABA levels reached a steady-state level between 12 and 24 h. This result indicates that the level of free ABA present in wilted WT leaves after 12 h is sufficient to continue ABA-induced accumulation of the mRNAs. In addition, there was a differential response of the three genes to exogenously applied ABA (Fig. 2). Therefore, pLE4, pLE16, and pLE25 may represent genes which differ in their responsiveness to ABA.

Unlike the accumulation of the three drought-induced mRNAs, pLE8 mRNA accumulation decreased in response to prolonged water deficit after an initial increase (Fig. 3A). Because pLE8 mRNA accumulation is unaffected by ABA (Fig. 2), reduction in this transcript level during increasing periods of water deficit clearly demonstrates that some changes in gene expression, in response to drought stress, are independent of ABA accumulation.

In summary, we have presented evidence for the isolation of three cDNAs, pLE4, pLE16, and pLE25, which represent mRNAs that accumulate during drought stress and are regulated by endogenous ABA. Drought-induced expression of these mRNAs is rapid and correlates with the accumulation of endogenous ABA with increasing periods of drought stress. Further characterization of these cDNAs and the isolation of genes which encode the three mRNAs should provide insight into the manner in which ABA regulates gene expression in response to water deficit.

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