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Characterization of Expression of Drought- and Abscisic Acid-Regulated Tomato Genes in the Drought-Resistant Species *Lycopersicon pennellii*[1]

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A number of genes are induced by drought stress, and some of these genes are regulated by the plant hormone abscisic acid (ABA). In tomato (*Lycopersicon esculentum*), four genes have been identified and isolated that require elevated levels of endogenous ABA for expression: *le4*, *le16*, *le20*, and *le25*. To gain a better understanding of the role of these genes during stress, their expression has been studied in the drought-resistant relative of tomato, *Lycopersicon pennellii*. It was determined that homologous genes to all four of the *L. esculentum* genes were present in the *L. pennellii* genome. Studies were undertaken to compare the expression characteristics of these genes in *L. esculentum*, *L. pennellii*, and their F_1. Using two methods of water-deficit imposition, whole plants to which water was withheld and detached leaves that were wilted to 88% of their original fresh weight, it was demonstrated that transcripts of these genes accumulated in *L. pennellii* in response to water deficit. In general, the increase occurred after a longer period of water deficit in *L. pennellii* than in tomato. As in drought-sensitive species, ABA levels were elevated by drought stress in *L. pennellii*, although the levels were reduced compared with those in tomato. All four tomato genes were responsive to ABA in *L. esculentum* and the F_1, but only three of the four genes (*le16*, *le20*, and *le25*) were induced in response to exogenous application of ABA in *L. pennellii*. The patterns of expression of these genes in *L. pennellii* are generally similar to that of *L. esculentum*; therefore, it is suggested that these genes play a similar, yet undefined, role in both genotypes rather than being genes that are responsible for the greater drought resistance of *L. pennellii*.

During drought stress plants experience a number of physiological and metabolic changes, including altered expression of many genes (Skriver and Mundy, 1990; Bray, 1991), and a concomitant increase in the concentration of the plant hormone ABA (Zeevaart and Creelman, 1988). The ABA that accumulates during drought stress is required for several of the changes in gene expression that occur during drought stress in tomato (*Lycopersicon esculentum*) leaves (Bray, 1988). Four tomato genes that are drought induced, *le4*, *le16*, *le25*, and *le20*, have been characterized (Bray et al., 1990; Cohen et al., 1991; Plant et al., 1991; our unpublished data). An ABA-deficient mutant of tomato, *flacca*, was used to demonstrate that these genes require elevated levels of ABA for expression (Cohen and Bray, 1990). However, the role these genes play in adaptation to stress is not understood. Adaptive responses result in improved performance of the plant during stress and likely involve alterations in enzyme activities and/or gene expression. Genes that confer drought tolerance have not been identified or isolated from any plant.

A wild relative of tomato, *Lycopersicon pennellii*, is capable of growing and reproducing with a minimal amount of water and resisting wilt in its native environment (Yu, 1972; Rick, 1973). Yu (1972) originally showed that *L. pennellii* requires less water than tomato and has a greater water use efficiency when described in terms of water consumed per gram fresh weight growth. These results were later confirmed by Martin and Thorstenson (1988) using carbon isotope composition and season-long water use efficiency measurements. *L. pennellii* can produce a fertile interspecific hybrid with *L. esculentum* when it is used as the pollen parent. In addition, members of the genus *Lycopersicon* contain homosequential chromosomes (Rick and Yoder, 1990), and the majority of cDNAs isolated from tomato have homologs in *L. pennellii* (Zamir and Tanksley, 1988). Therefore, *L. pennellii* could be expected to have homologs of the four *le* genes described for tomato.

One means to evaluate the significance of the expression of specific genes with respect to stress tolerance is to compare the pattern of expression in different genotypes. If drought-induced genes are differentially expressed in drought-resistant and drought-sensitive species, one could judge if these genes have an adaptive role. Using this strategy, the expression of several drought-induced genes has been characterized in three genotypes: cultivated tomato, which is relatively drought sensitive; a closely related drought-resistant wild species, *L. pennellii*; and their interspecific hybrid.

MATERIALS AND METHODS

Plant Material

Tomato (*Lycopersicon esculentum* Mill. cv UC82L), *Lycopersicon pennellii* Corr. LA716, and the interspecific hybrid (tomato × *L. pennellii*; F_1) were grown from seed or cuttings in either the greenhouse or growth chamber as specifically

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indicated. Unless otherwise described, plants were kept well watered and fertilized with Stern's Miracle Grow or half-strength Hoagland solution (Hoagland and Arnon, 1938). Growth chamber conditions were 16-h d at 25°C.

Experimental Treatments

Extended Stress Treatment

For long-term drought-stress treatments, whole plants were grown in either soil mix or sand in the greenhouse with daily watering. When the plants were several weeks old, water was withheld until they were visibly wilted (1–5 d, depending on genotype and plant size relative to pot size). Young leaves from wilted plants were collected into liquid nitrogen, and all the roots from wilted, young plants grown in sand were collected into liquid nitrogen. Control, nonstressed root or leaf samples were collected into liquid nitrogen from plants watered daily with no visible wilt symptoms. For the time course study, plants were seeded in 15-cm pots. When the plants were 6 weeks old, water was withheld and leaf samples were collected into liquid nitrogen at 12-h intervals, until the plants wilted.

Short-Term Stress Treatment

For short-term water deficits, experiments were conducted on expanding tomato leaves that were detached from the plant and immediately placed in water. Leaves were then wilted to 88% of their original fresh weight on the laboratory bench within approximately 0.5 h and maintained in their wilted state in clear plastic bags for the duration of the treatment. For time course studies, leaves were weighed, wilted, then placed in bags for periods of 1 to 24 h, measured from the start of wilting. Nonstressed leaves were placed in water for the treatment period. ABA treatments were applied to leaves by placing petioles in a solution of 0.01, 0.1, or 1 mM (±)-ABA (Sigma) for 6 h. Leaflets were frozen in liquid nitrogen and stored at −80°C.

Nucleic Acid Isolation and Analyses

Total DNA was isolated from fresh leaf tissue as described by Doyle and Doyle (1989), with the addition of a CsCl centrifugation after the isopropanol precipitation. DNA (10 μg) was digested with restriction enzymes for at least 4 h at 37°C; electrophoresis, DNA transfer, and hybridization conditions were as described by Melzer et al. (1989), with the exception of the low-stringency wash conditions, which were 3× SSC.

Extended Stress Treatment

Total RNA was isolated as described by Cohen and Bray (1990), including an additional LiCl precipitation step from frozen leaf and root samples obtained from extended stress plants. RNA was size-fractionated in formaldehyde denaturing 1.2% agarose gels (Sambrook et al., 1989). Ethidium bromide (1 μg) was added to each sample, and following electrophoresis the gel was photographed under UV light to demonstrate the quality of the RNA and to confirm that an equivalent amount of RNA was loaded in each lane. RNA was transferred to nitrocellulose or Zetabind membranes, and prehybridization, hybridization, and washing of membranes were carried out as described by Melzer et al. (1989). Probes for DNA and RNA blots were synthesized by oligolabeling (Feinberg and Vogelstein, 1983) using gel-purified inserts of the indicated pLE cDNA clones, or in the case of pLE4, an *Fok*I fragment of the cDNA insert was end-labeled (Sambrook et al., 1989). The *Fok*I fragment of pLE contained a region common among other homologs of this gene (Cohen et al., 1991). This DNA fragment is designated pLE4f.

Short-Term Stress Treatment

For the short-term stress experiments, total RNA was extracted from tomato leaflets (Cohen and Bray, 1990). Thirty micrograms of total RNA was size-fractionated in a formaldehyde denaturing 1.5% agarose gel according to Sambrook et al. (1989). The RNA was transferred to Zeta-probe membranes (0.45 μm; Bio-Rad) using 20× SSC as the transfer buffer. Following transfer, RNA was immobilized by cross-linking with UV irradiation at 300 nm for 3 min. Membranes were prehybridized and hybridized with antisense RNA probes according to Cohen and Bray (1990). RNA blots were hybridized overnight at 60°C in 10 mL of prehybridization solution with the addition of 3×10^7 cpm of antisense RNA probes to pLE16, pLE20, and pLE25. The antisense RNA probe for pLE4 was to a 150-bp *Fok*I fragment of pLE4 also designated pLE4f. Membranes were washed and stained according to procedures outlined by Cohen et al. (1991).

Quantitation of ABA

ABA was quantified using a competitive ABA radioimmunoassay as described in detail by Bray and Beachy (1985).

Determination of Water Potentials

A J.R.D. Merrill thermocouple psychrometer (Logan, UT) was used to determine leaf water potentials of drought-stressed, detached leaves. Detached leaves from each of three genotypes were stressed as above, then 6-mm leaf discs were cut from leaves and two discs were quickly transferred to each thermocouple psychrometer. All thermocouple psychrometers used in these experiments were individually calibrated with a range of Suc solutions of known molarity. To determine the equilibrium values for total and osmotic potentials, samples were placed in a covered 29°C water bath, and 10 wet bulb readings were recorded every hour over 20- to 26-h periods with a Campbell CR7 datalogger (Logan, UT). Osmotic potentials were determined after freezing and thawing the same samples in liquid nitrogen, and assuming negligible dilution by apoplastic water. Turgor potentials were calculated by subtracting osmotic potential from total water potential.

RESULTS

Identification of *L. pennellii* Homologs to *le* Genes

Genomic DNA hybridizations indicate that homologs of the four drought- and ABA-induced tomato genes are present

in the *L. pennellii* genome. DNA blot analyses of genomic DNA isolated from the tomato cultivar UC82L and *L. pennellii* were performed using the four cDNAs, pLE4, pLE16, pLE20, and pLE25, for hybridization (Fig. 1). To detect hybridization of pLE4 to *L. pennellii*, a 150-bp *Fok*I fragment containing the carboxy terminus of the coding sequence was used for hybridization (pLE4f) and washes were performed under low stringency. This region of the gene product is highly conserved among all members of this homology group (Cohen et al., 1991; Galau and Close, 1992). Two DNA fragments, one of 13.2 kb and another of 9.5 kb, hybridize with pLE4f in *Hin*dIII digests of tomato DNA, whereas one 16-kb fragment of *L. pennellii* DNA hybridizes with pLE4f (Fig. 1). When pLE20 or pLE25 was hybridized with *Hin*dIII-digested DNA, in each case only one fragment was observed to hybridize to each genotype: a 2.4-kb DNA fragment of tomato and 6.0 kb of *L. pennellii* hybridized to pLE20, and a 1.4-kb DNA fragment of tomato and 4.7 kb of *L. pennellii* hybridized to pLE25 (Fig. 1). Similar results were observed for these three cDNAs with other restriction enzymes; usually only one fragment was observed to hybridize (data not shown). These results are consistent with the hypothesis that *le4*, *le20*, and *le25* are present in both genotypes as single-copy genes. One prominent DNA fragment from *Hin*dIII-digested DNA hybridized with pLE16 in both genotypes: 4.2 kb in tomato and 2.3 kb in *L. pennellii* (Fig. 1). A longer exposure of the image in Figure 1 revealed multiple weaker bands in both genotypes, which may indicate that there are related genes in both genomes. The tomato genome is known to contain another gene related by DNA sequence to *le16*, TSW12, which is also regulated by abiotic stresses (Torres-Schumann et al., 1992). A genomic DNA fragment from *L. pennellii* has been isolated that contains two regions, approximately 2.5 kb apart, that hybridize to pLE16, one of which has been shown to contain an intact gene (M.B. Trevino, unpublished data). A similar contiguous organization for members of this gene family has been described in tobacco (Fleming et al., 1992). The intensity of hybridization of the tomato cDNA to *L. pennellii* DNA fragments is equivalent to the tomato signal for pLE20, approximately 50% of the intensity for pLE25, and less than 50% of the intensity for the other two, pLE4f and pLE16.

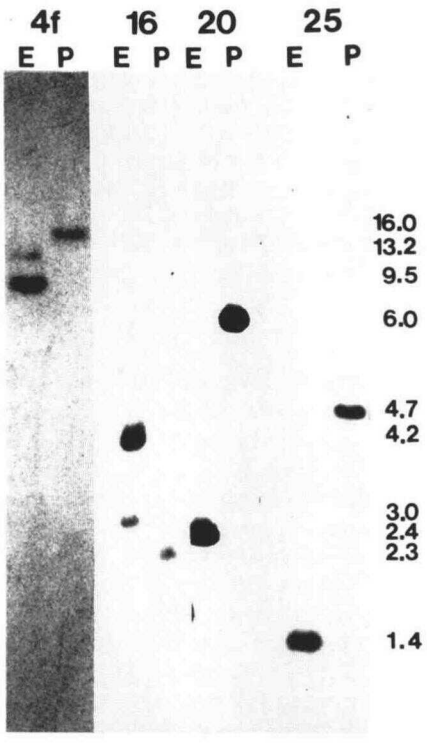

Figure 1. Southern hybridizations of genomic DNA from tomato or *L. pennellii*. Total DNA isolated from either tomato cv UC82L (E) or *L. pennellii* (P) was digested with *Hin*dIII. DNA blots of the digests were probed with end-labeled *Fok*I fragment from pLE4 (4f) or oligolabeled inserts from the cDNA clones pLE16 (16), pLE20 (20), or pLE25 (25). Sizes of the hybridizing fragments are indicated in kb.

Expression of *le4*, *le16*, *le20*, and *le25* in Leaves and Roots Subjected to Extended Stress Treatments

To determine if the drought-induced genes of tomato are also drought induced in *L. pennellii* and the interspecific F_1, RNA was isolated from young leaves and roots of drought-stressed plants. RNA blots were performed using the cDNAs, pLE4, pLE16, pLE20, and pLE25, for hybridization. Only weak hybridization to *L. pennellii* DNA was observed when the entire pLE4 cDNA sequence was used in hybridization (data not shown). Therefore, pLE4f was used for hybridization.

All four RNAs accumulated in leaves of tomato plants that were drought stressed (Figs. 2-4). In the F_1, all four RNAs also accumulated in drought-stressed leaves relative to nonstressed leaves. Transcripts for *le16*, *le20*, and *le25* also accumulated in response to drought relative to control in *L. pennellii* leaf tissue. However, no RNA was observed to hybridize to pLE4f in control or drought-stressed *L. pennellii* shoot tissue (Figs. 2 and 3). Overall, among the three genotypes, drought-stressed F_1 individuals had the greatest hybridization of pLE16, pLE20, and pLE25 to transcripts isolated from leaves, followed by drought-stressed tomato and drought-stressed *L. pennellii* (Fig. 2).

To determine if the roots of drought-stressed plants also accumulated these transcripts, RNA was isolated from roots of nonstressed or drought-stressed tomato, *L. pennellii*, or the F_1, and RNA blots were hybridized with the pLE cDNAs (Fig. 2). Transcripts for *le4* and *le25* accumulated in response to drought in roots of all three genotypes. No RNA isolated from roots of nonstressed or drought-stressed plants was observed to hybridize to pLE16 in any genotype. Transcripts for *le20* were present in both nonstressed and drought-stressed roots of all three genotypes. In general, the pattern of accumulation with respect to water deficit and organ of expression was similar in all genotypes.

Accumulation of Drought-Induced mRNAs during Imposition of Drought

The time it took for plants to visibly wilt after water was withheld was quite different among the three genotypes.

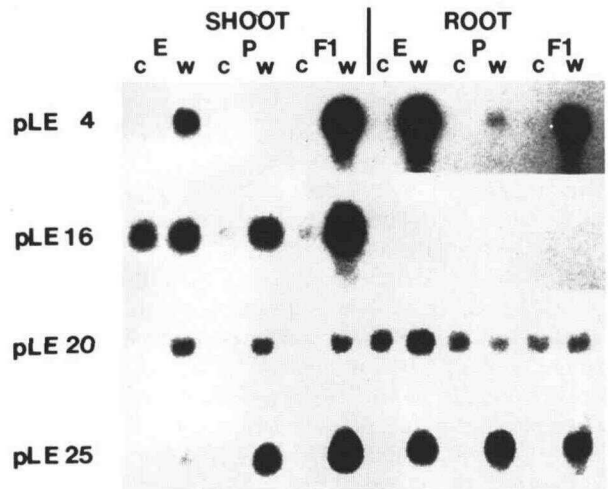

Figure 2. Drought-induced accumulation of *le4*, *le16*, *le20*, and *le25* transcripts in shoot and root tissues of three tomato genotypes. Total RNA was isolated from shoots or roots of tomato cv UC82L (E), *L. pennellii* (P), or the interspecific hybrid (F_1) plants that were well watered (c) or subjected to drought until visibly wilted (w). Following electrophoresis of 25 μg of RNA and transfer to nylon membranes, the blots were hybridized with oligolabeled cDNA insert from pLE16, pLE20, or pLE25 or the end-labeled *Fok*I fragment of pLE4.

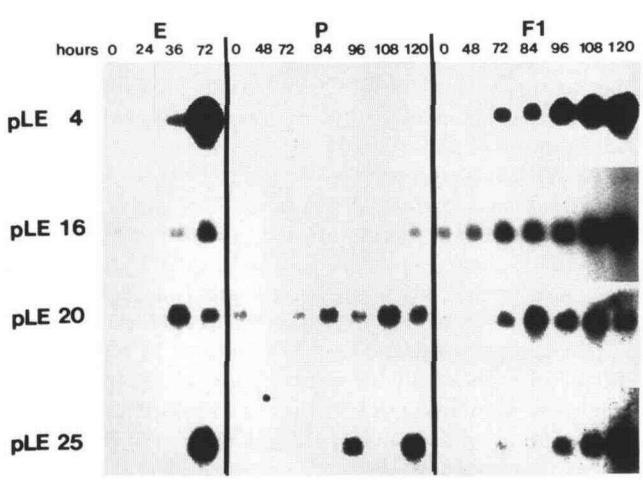

Figure 3. Time course of the accumulation of *le4*, *le16*, *le20*, and *le25* transcripts in shoot tissues of three tomato genotypes during the imposition of drought. Total RNA was isolated from shoots of tomato cv UC82L (E), *L. pennellii* (P), or the interspecific hybrid (F_1) at the indicated times following the imposition of drought. Following electrophoresis of 25 μg of RNA and transfer to nylon membranes, the blots were hybridized with oligolabeled cDNA insert from pLE16, pLE20, or pLE25 or the end-labeled *Fok*I fragment of pLE4.

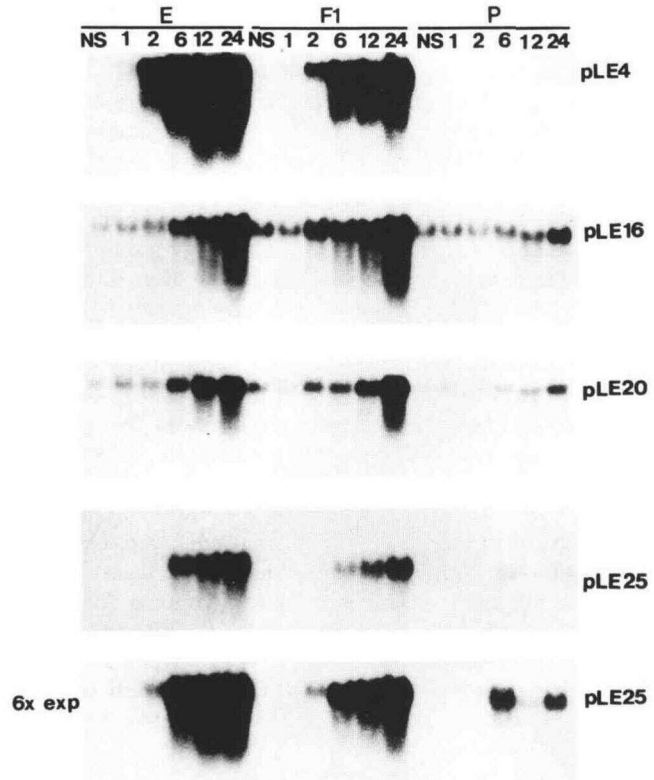

Figure 4. Time course of the accumulation of *le4*, *le16*, *le20*, and *le25* transcripts in detached leaves wilted to 88% of their fresh weight and maintained in plastic bags from 1 to 24 h. Total RNA was isolated from the leaves that had been stressed for 1, 2, 6, 12, or 24 h or leaves that were not stressed (NS) of the three genotypes, tomato (E), the interspecific hybrid (F_1), and *L. pennellii* (P). Following electrophoresis of 30 μg of RNA and transfer to nylon membrane, the blots were hybridized with ^{32}P-labeled antisense RNA synthesized from the cDNAs, pLE4f, pLE16, pLE20, and pLE25. A longer exposure of the blot hybridized with pLE25 is also shown (6× exp).

Tomato plants were visibly wilted at 72 h, F_1 plants at 108 h, and *L. pennellii* plants at 120 h. To determine if the accumulation of drought-induced transcripts was correlated with visible wilting, RNA was isolated from leaves of plants at timed intervals following the imposition of drought stress. Water was withheld from well-established plants of equivalent age of all three genotypes (t = 0). At 0, 24, 36, 48, 72, 84, 96, 108, and 120 h leaves were collected for RNA isolation and the physical appearance of the plants was noted. Since young and mature leaves were used for the different time points, the effect of development on expression of these sequences was investigated. RNA was isolated from young and mature leaves of drought-stressed individuals of all three genotypes. The older leaves appeared to be more wilted than the younger leaves; however, no differences in hybridization signal were observed as a function of development for any of the *le* genes (data not shown).

The RNA isolated from the three tomato genotypes at different times during the imposition of drought was size-fractionated, transferred to membranes, and then hybridized

with the four cDNAs (Fig. 3). The mRNAs for *le4* and *le16* were first detected in tomato at 36 h and were abundant at 72 h, the time point at which the plants were observed to be wilted. The *le25* transcript was only detected at 72 h in tomato, which coincided with wilting. The accumulation of transcripts for *le20* was maximal at 36 h in tomato, prior to visible wilting. The accumulation of all four drought-induced mRNAs in the F_1 preceded the appearance of wilt in the plant, although maximum amounts of the transcripts were observed at the time of visible wilt, 108 to 120 h. A different pattern of accumulation was observed in *L. pennellii* plants; the *le16* transcript was detected only in visibly wilted plants, and the *le4* transcript was not detected. *le20* mRNA in *L. pennellii* was detected after 72 h of stress and was at the maximum level at 108 h. The pattern of *le25* accumulation, present at 96 and 120 h and undetectable at 108 h, was reproducibly observed. This same RNA blot was hybridized with a rRNA sequence, and, as expected based on the ethidium bromide staining of this gel, all lanes on the blot hybridized equivalently with the rRNA probe (data not shown). The increase and decrease in *le25* mRNA during stress was unique to *L. pennellii*.

Overall, the accumulation of *le20* mRNA preceded visible wilt in all three genotypes, whereas the accumulation of *le25* mRNA most closely paralleled the visible state of the plant in all three genotypes. The accumulation patterns of *le4* and *le16* mRNAs were dependent on the genotype; in tomato, these transcripts accumulated to significant levels at the time of wilt, and in the F_1 there were significant quantities of these transcripts in plants that did not appear wilted. In *L. pennellii*, *le16* mRNA was detected only in visibly wilted plants, and no *le4* mRNA was detected.

Accumulation of *le4*, *le16*, *le20*, and *le25* mRNA in the Different Genotypes after Short-Term Stress Treatments

The pattern of RNA accumulation in detached leaves was characterized in order to observe a short-term stress of leaves that had lost the same percentage of water. RNA was isolated from detached leaves, which had been wilted to 88% of their fresh weight, of the three genotypes (Fig. 4). *le16* and *le20* mRNAs were detected in nonstressed leaves of all three genotypes. In tomato and the F_1, the level increased after 1 h and continued to accumulate through 24 h (Fig. 4). The amount of hybridization to pLE16 and pLE20 was similar in tomato and the F_1. However, the pattern of accumulation of *le16* and *le20* mRNAs was different in *L. pennellii*. Both *le16* and *le20* mRNAs were detected in nonstressed *L. pennellii* leaves, but *le16* mRNA accumulation was not elevated over nonstress levels until after 12 h, and *le20* mRNA accumulation increased above nonstress levels after 6 h.

le4 mRNA was first detected at 1 h after stress and accumulated over 24 h in both tomato and the F_1, with the greatest accumulation appearing in tomato. Hybridization of *L. pennellii* mRNA to pLE4f was detected after drought stress only after a long exposure of the blot. The mRNA level was similar after 6, 12, and 24 h of water deficit (data not shown). Hybridization to *le25* mRNA was first observed after 2 h of stress in tomato and the F_1; the greatest accumulation was at the 24-h stress time point. In *L. pennellii* the mRNA level was elevated at 6 and 24 h and decreased at 12 h. In the F_1 *le25* mRNA was detected after 2 h.

In all cases hybridization of tomato cDNAs to *L. pennellii* mRNA was reduced compared with that of tomato or F_1 mRNA, and the pattern of accumulation of the mRNAs was similar in tomato and the F_1. The accumulation of the mRNAs was drought induced in *L. pennellii*, but the patterns were different from tomato and the F_1. For *le16* and *le20* the mRNAs were at nonstress levels for 12 h and then increased at 24 h; therefore, in *L. pennellii* the mRNAs accumulated at a later time point. *le4* mRNA was not detected in *L. pennellii* and *le25* mRNA was increased at 6 and 24 h relative to nonstress, but not at 12 h of drought stress.

ABA Concentration in Leaves of Tomato, *L. pennellii*, and Their F_1 after Short-Term Stress Treatments

The four genes are induced by elevated levels of endogenous ABA in tomato, and the amount of the mRNAs corresponds with the concentration of ABA in the leaves (Cohen and Bray, 1990; our unpublished data). The level of ABA in detached leaves was determined at different time points for each of the three genotypes over a 24-h period of water deficit. All three genotypes responded to drought stress with respect to ABA accumulation, although there were significant differences among genotypes in the concentration and in the pattern of ABA accumulation (Fig. 5). Prior to stress, the ABA level of tomato leaves was the greatest. The level of ABA in *L. pennellii* was one-fifth that of tomato and the F_1 was intermediate to its parents. During drought stress the leaf ABA level of tomato steadily increased to 3518 ng/g fresh weight at 24 h, a 7.5-fold increase. The ABA level of *L. pennellii* also increased during the time course to 643 ng/g fresh weight, remaining approximately one-fifth that of tomato, a 6.5-fold increase. The overall increase in ABA was 13.5-fold in the F_1, which was a greater increase than in either of its parents.

The ABA levels in the F_1 were similar to those in tomato at 24 h; however, the pattern of ABA accumulation in the F_1 leaves was different from tomato and *L. pennellii*. ABA levels increased rapidly in response to water deficit in all of the

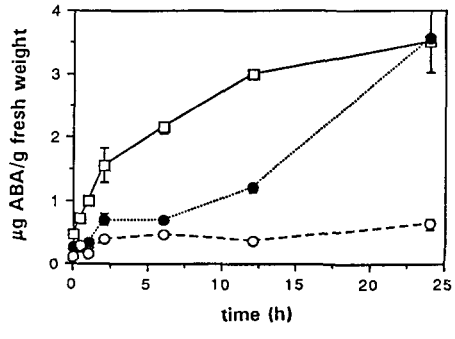

Figure 5. ABA concentration of detached leaves that were wilted to 88% of their original fresh weight and maintained in plastic bags for the times indicated. The ABA concentration in leaves from three genotypes, tomato (□), the interspecific hybrid, F_1 (●), and *L. pennellii* (○), was measured using a radioimmunoassay.

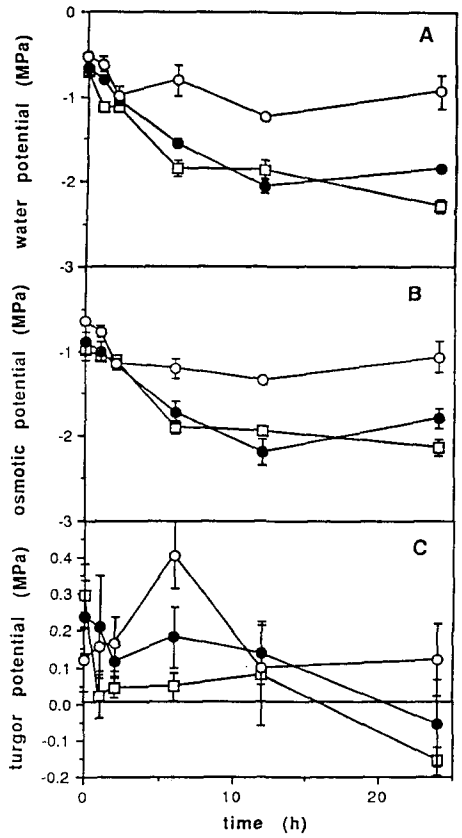

Figure 6. Water potential of detached leaves that were wilted to 88% of their original fresh weight and maintained in plastic bags for the times indicated. Water potentials were determined with a thermocouple psychrometer on three genotypes: tomato (□), the interspecific hybrid, F_1 (●), and *L. pennellii* (○). A, Total water potential was determined on leaf discs; B, osmotic potential was determined on the same leaf discs after freezing and thawing; C, turgor potential was derived from the above data.

genotypes. An increase in ABA was measured 30 min after imposition of water deficit in tomato and *L. pennellii*. In tomato the level of ABA increased steadily, whereas in *L. pennellii* the level increased gradually through 2 h, remained level through 12 h, then increased again at 24 h. In the F_1 the level of ABA increased gradually through 2 h, and from 6 h to 24 h.

Leaf Water Potentials of the Different Genotypes after Short-Term Stress Treatments

Water potentials of detached leaves of tomato, *L. pennellii*, and their F_1 decreased in response to drought stress (Fig. 6). However, the magnitude of decrease differed among the three genotypes. The leaf water, osmotic, and turgor potentials of nonstressed, detached leaves were similar in all three genotypes. At 1 h after wilting to 88% of original fresh weight, there was an appreciable decrease in the leaf water potential of detached leaves of tomato (Fig. 6A). The same water potential occurred 2 h after wilting in the F_1 and 12 h after wilting in *L. pennellii*. The minimum mean leaf water potential in tomato was −2.2 MPa at 24 h after wilting, which was significantly different than that of *L. pennellii*, which reached −1.2 MPa at 12 h after wilting. The pattern of leaf water potential changes in the F_1 was more similar to that of tomato than that of *L. pennellii*. The changes in osmotic potential over time followed the changes in leaf water potential in all three genotypes (Fig. 6B).

The mean turgor potential of detached leaves of tomato dropped from 0.29 to 0.02 MPa at 1 h after wilting and remained near zero until 24 h, when the turgor was calculated to be −0.16 MPa (Fig. 6C). In contrast, the mean turgor potential of *L. pennellii* nonstressed, detached leaves was 0.12 MPa, then increased to 0.4 MPa at 6 h after wilting, then dropped to near 0.1 MPa at 12 and 24 h after wilting. Mean turgor potential of nonstressed F_1 leaves was 0.2 MPa and remained between 0.2 and 0.1 MPa, and was then calculated to be −0.5 MPa 24 h after drought stress.

Induction of Gene Expression in Response to Applied ABA

To determine if the homologs of ABA-induced tomato genes respond to ABA treatments in *L. pennellii*, detached leaves were fed 0.01, 0.1, and 1 mM ABA. The ABA concentrations and specific mRNA levels were compared among the genotypes (Table I, Fig. 7). After 0.01 mM ABA application there was a 3-fold difference in ABA concentration among the genotypes (Table I). The ABA level in *L. pennellii* was 715 ng ABA/g fresh weight, which was approximately equal to a 0.5-h drought-stress treatment in tomato and a 24-h drought-stress treatment in *L. pennellii* (Fig. 5). After 0.1 mM ABA application there was a 5-fold difference in ABA concentration between tomato and *L. pennellii*. The level of ABA was greatest in tomato, where it was equal to the level achieved after 24 h of drought stress. After 1 mM ABA application the ABA concentration in tomato was more than 56-fold greater than the 0.1-mM application in tomato. In addition, the level of ABA in tomato leaves treated with 1 mM ABA was 5- and 7-fold greater than *L. pennellii* and the F_1, respectively. This level of ABA in tomato leaves was 60-fold greater than the ABA levels achieved after a 24-h drought stress of detached leaves.

To determine if the genes were induced by ABA application in all of the genotypes, RNA was isolated from detached leaves that were fed ABA, then RNA blots were prepared and hybridized with each of the cDNAs (Fig. 7). In general, the hybridization to RNA was greatest for tomato and intermediate for F_1, and the least hybridization was detected for

Table I. *Concentration of ABA in leaflets after ABA application*

ABA was applied to detached leaves of the three genotypes for 6 h, after which ABA levels were determined by radioimmunoassay.

Genotype	Concentration of Applied ABA		
	0.01 mM	0.1 mM	1 mM
	μg ABA/g fresh weight		
Tomato	1.16 ± 0.34	3.66 ± 0.25	208.71 ± 28.36
F_1	0.33 ± 0.18	2.70 ± 0.10	30.19 ± 3.62
L. pennellii	0.71 ± 0.15	0.77 ± 0.17	37.11 ± 20.26

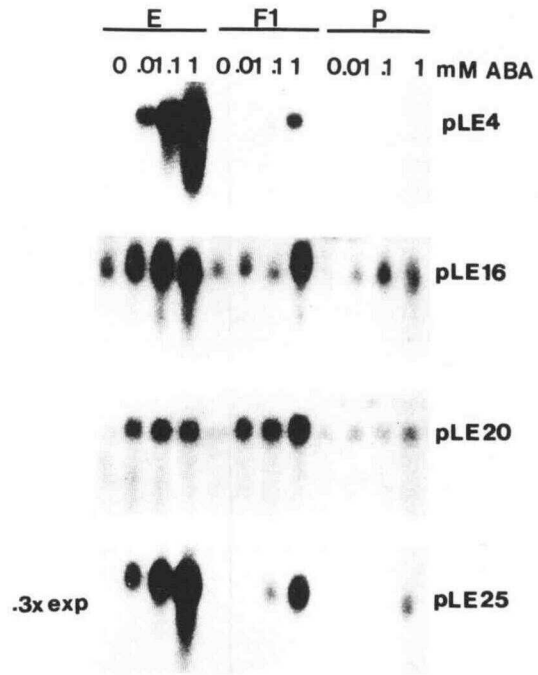

Figure 7. Accumulation of *le4*, *le16*, *le20*, and *le25* mRNA in response to ABA application. Total RNA was isolated from the three different genotypes, tomato (E), the interspecific hybrid (F_1), and *L. pennellii* (P), after detached leaves were treated with ABA (0.01, 0.1, and 1 mM) or maintained in water (0) for 6 h. RNA was separated by denaturing agarose gel electrophoresis and blotted to nylon membrane and hybridized with ^{32}P-labeled antisense RNA synthesized from the cDNAs pLE4f, pLE16, pLE20, and pLE25. The blot hybridized to pLE25 was exposed to film for one-third the time of the other blots (.3× exp).

L. pennellii. The *le4* and *le25* mRNA levels increased with greater concentration of applied ABA. The hybridization to *le4* and *le25* mRNAs was greatest in tomato, followed by the F_1 and then *L. pennellii*. *le4* and *le25* were detected after all three levels of applied ABA in tomato. However, *le4* and *le25* mRNAs were only detected in F_1 leaves treated with 0.1 and 1 mM ABA. *le25* mRNA was detected in *L. pennellii* after 1 mM ABA application, but *le4* mRNA was not detected in *L. pennellii* after any concentration of applied ABA, even after prolonged exposure of the blot to film (data not shown). *le16* and *le20* mRNA accumulation was the same in tomato at all three concentrations of ABA. However, in leaves of the F_1 the greatest accumulation of *le16* and *le20* mRNA was found after treatment with 1 mM ABA, and the hybridization was similar to the maximum level observed in drought-stressed tomato leaves. In *L. pennellii* leaves *le16* and *le20* mRNA was detected at all levels of applied ABA, but hybridization was greatest in response to 0.1 and 1 mM ABA for *le16* mRNA and to 1 mM ABA for *le20* mRNA.

DISCUSSION

Drought stress causes many changes in gene expression. A number of genes that preferentially accumulate in response to water deficit have been isolated and characterized (Close et al., 1989; Skriver and Mundy, 1990). Among these are four tomato genes that are induced by drought and ABA in this drought-sensitive species (Bray et al., 1990; Cohen and Bray, 1990; Cohen et al., 1991; Plant et al., 1991). *L. pennellii* is a drought-resistant relative of tomato with a genome structure similar to tomato (Zamir and Tanksley, 1988; Rick and Yoder, 1990). Our results demonstrate that the *L. pennellii* genome contains homologs of each of the drought-induced genes studied in tomato. One gene, *le20*, hybridized with equal intensity to tomato and *L. pennellii*. The other genes hybridized with less intensity to *L. pennellii*. The hybridization patterns for *le4*, *le20*, and *le25* are consistent with the hypothesis that these genes are in the tomato and *L. pennellii* genome in one copy. In tomato, a gene that is similar to *le16* has been reported; TSW12, which has also been shown to be induced during environmental stress, is similar to *le16* in deduced amino acid sequence (Torres-Schumann et al., 1992). It is likely that there is also more than one *le16*-like gene in *L. pennellii*. Two independent regions of hybridization to pLE16 were identified on an *L. pennellii* genomic DNA fragment (M.B. Trevino, unpublished data). These results are consistent with those of tobacco in which two *le16*-like genes have also been identified (Fleming et al., 1992).

A comparison of patterns of expression of these genes among the two related genotypes that differ in drought resistance can be used to further characterize the functional roles of the gene products. As in tomato, the homologs of the *le* genes were induced by water deficit in the drought-resistant species *L. pennellii* and the interspecific hybrid. To quantify the relative abundances of the transcripts in the different genotypes, the four radiolabeled cDNAs used for hybridization must have similar specific activities, and the DNA sequences of *L. pennellii* and tomato must hybridize equally to the cDNAs. However, only pLE20 hybridized equally to genomic DNA of tomato and *L. pennellii*; therefore, quantitative comparisons among the genotypes can only be made for *le20*.

In general, accumulation of the mRNAs occurred after longer periods of water deficit in *L. pennellii* than in tomato or the F_1 in both extended-stress and short-term stress treatments. In the extended stress experiments, water was withheld from plants until wilting was visible, and it took longer for *L. pennellii* plants to wilt. The duration required for accumulation of the transcripts corresponded with the time to wilting.

When the same percentage of water loss was imposed on the different genotypes in the short-term experiments, the resulting leaf water potential of tomato and the F_1 were similar; however, the water potential of *L. pennellii* was at a higher value. In another study, when plants were stressed at a single concentration of PEG, the leaf water potential of *L. pennellii* was higher than tomato (Pillay and Beyl, 1990). Differences among tomato, the F_1, and *L. pennellii* in water content of the plant tissue might explain higher water potentials in *L. pennellii* after the same percentage of water loss and PEG treatment. Yu (1972) demonstrated that *L. pennellii* tissue has the highest water content (93.9%), whereas tomato (90.0%) and the F_1 (89.5%) have a lower water content. These differences among the three genotypes are significant at the 0.1% level. In the short-term stress treatments, there was less

le20 mRNA in *L. pennellii* than in tomato and the F_1. And, an increase in response to water deficit in the other mRNAs relative to nonstress levels was detected later or was not detected at all in *L. pennellii* (Fig. 4). Therefore, the ability of the *L. pennellii* leaves to maintain a higher water potential than tomato may explain the differences in the accumulation of the mRNAs. The accumulation of these mRNAs in all of the genotypes studied may be dependent on the duration and the extent of the stress.

The genes of tomato used in this study require elevated levels of ABA for expression (Cohen and Bray, 1990). If there is a similar regulation pattern in the different genotypes, the *L. pennellii* genes would also be regulated by ABA, and an increase in ABA concentration in response to drought stress in *L. pennellii* would be expected. In the drought-resistant species, *L. pennellii*, ABA levels increased in response to drought stress. These results support the conclusion that ABA concentration is increased in drought-tolerant and -sensitive species during periods of water deficit. Studies of several resurrection plants known to be tolerant to desiccation also demonstrate that there is an increase in ABA in response to water deficit (Gaff and Loveys, 1984; Bartels et al., 1990). However, the level of ABA in *L. pennellii* after 24 h of drought stress was less than in tomato or the F_1.

During the imposed stress, turgor pressure did not reach zero in *L. pennellii* as it did in tomato and the F_1. This may account for the genotypic differences in ABA levels in response to water deficit, because increased production of ABA occurs as turgor approaches zero during periods of decreasing water potential (Pierce and Raschke, 1980). In detached leaf studies of ABA accumulation during water stress in mature leaves of *Phaseolus vulgaris*, *Xanthium strumarium*, and *Gossypium hirsutum*, Pierce and Raschke (1980) found that, below turgor pressures of 0.1 MPa, ABA content increased sharply to as much as 40 times the level found in unstressed samples. Accumulation of some ABA before the point of zero turgor occurs because a leaf consists of a heterogeneous population of cells with different water and turgor potentials (Zeevaart and Creelman, 1988). During water deficit in detached leaves of the three genotypes, the turgor declined below 0.1 MPa in tomato first, which coincided with the most rapid increase in ABA concentration. The turgor of the F_1 did not drop below 0.1 MPa until 24 h, which coincided with the largest increase in ABA. The turgor of *L. pennellii* did not decrease below 0.1 MPa, and the ABA concentration was the lowest. The pattern and amount of ABA accumulation was related to the leaf water relations.

Transcripts of all four genes in tomato accumulate in response to ABA. Three of the tomato genes, *le16*, *le20*, and *le25*, are also responsive to ABA in *L. pennellii*. Like tomato, genes can be induced by ABA in the drought-tolerant species, *L. pennellii*. *le4* mRNA was not detected in response to exogenous applications of ABA in *L. pennellii*. The patterns of hybridization indicate that *le4* and *le25* require a threshold level of ABA for expression and that the level of ABA required may be different for all of the genotypes (Fig. 7). Hybridization to *le16* and *le20* mRNAs was detected at the lowest level of ABA applied in all genotypes. Therefore, ABA must be applied at a lower concentration to determine whether there is a threshold level of ABA required for expression of these genes and whether it is the same in all three genotypes. In general, the level of accumulation of each mRNA corresponded to the ABA concentration measured in ABA-treated leaves. The pattern of ABA and mRNA accumulation was also similar in detached leaves subjected to water deficit. Although these experiments cannot determine whether ABA is an endogenous regulator of specific mRNA accumulation in *L. pennellii*, these results are consistent with that role.

Many genes that are induced by drought are responsive to ABA (Skriver and Mundy, 1990; Bray, 1991). The majority have been studied in relatively drought-sensitive crop species. However, these studies and those of the resurrection plant indicate that ABA may also play a role in the induction of drought-induced genes in species that tolerate water deficit. The resurrection plant, which can withstand desiccation, accumulates a number of mRNAs in response to water deficit. These genes are also induced by ABA treatment (Bartels et al., 1990).

Although many investigators have identified genes whose abundance increases in response to abiotic stresses, very few have investigated the expression of these same genes in genetically related plants that differ in abiotic stress resistance. The documented drought resistance of the wild tomato species, *L. pennellii*, was exploited to determine whether any of the drought-induced genes isolated from the relatively drought-sensitive species, cultivated tomato, were differentially expressed. Because adaptive responses result in a greater ability of the plant to withstand stress, alterations in gene expression that confer this response would be different in drought-sensitive and drought-resistant species. The differences may be in the regulation of expression of a specific gene(s) or in the functional characteristics of a specific gene product(s). The experiments reported demonstrate that in *L. pennellii* homologs of tomato genes are regulated in a similar manner to tomato, indicating that the mechanism of induction in *L. pennellii* is similar to that in tomato. The transcript levels are increased in response to drought in both species, depending on the extent and duration of the stress. None of the four *le* genes are expressed constitutively in the drought-resistant species *L. pennellii*. Therefore, it is unlikely that the timing of expression of these genes is responsible for the difference in drought resistance of these genotypes. Specific functions of the different drought-induced genes are not known, and, therefore, it cannot be determined whether the gene products from the different genotypes have different functional characteristics that are involved in adaptation. It is likely that numerous genetically based traits, including slower growth rate, thicker leaves, fewer stomata, and a higher percentage of water in plant tissue of *L. pennellii* than in tomato, contribute to the drought resistance of *L. pennellii*.

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