Tissue-specific Expression of a Dehydrin Gene in One-year-old 'Rio Oso Gem' Peach Trees

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ABSTRACT. Dehydrins are desiccation-induced proteins. Many plants have several dehydrin genes, some of which are primarily cold induced while others are primarily abscisic acid (ABA) or desiccation induced. Only one dehydrin gene (*ppdhn1*) has been reported in peach. The dehydrin gene is seasonally regulated and associated with cold acclimation. Because molecular markers for desiccation resistance may aid in the selection of drought- and cold-tolerant genotypes, we sought to determine if *ppdhn1* was inducible by desiccation and ABA in all tissues (i.e., a whole-plant response) and to examine the relationship between expression of *ppdhn1*, desiccation, and dehydrin protein (PCA60). One-year-old 'Rio Oso Gem' peach [*Prunus persica* (L.) Batsch.] trees were maintained at a stem water potential of -2.0 MPa by withholding water for 1 week, followed by daily watering for 1 week for some of the trees. ABA (100 mM) was applied to similar trees that were well watered. Total RNA and protein were extracted from bark, leaves, xylem, and roots, fractionated by electrophoresis, blotted to membranes, and probed with either a peach-specific dehydrin cDNA clone or polyclonal antibodies directed against dehydrin. Accumulation of *ppdhn1*/PCA60 was induced more by desiccation than ABA applications. Additionally, such accumulation was tissue dependent, being highest in bark tissues and lowest in leaf tissues. The presence of *ppdhn1* transcript and corresponding PCA60 protein were not always commensurate with each other. In particular, elevated levels of PCA60 were still present 1 week after desiccation recovery when transcript levels had decreased significantly or were undetectable, indicating that dehydrin is a stable protein. In general, our data indicate that *ppdhn1* is similar to other cold-induced dehydrins that are only slightly induced by ABA. In contrast to cold-induced dehydrins, *ppdhn1* was strongly induced by desiccation. While synthesis of dehydrin is tightly associated with the onset of stress, disappearance and turnover seem less linked to alleviation of the inducing stress.

Plant responses to environmental stresses are reflected in the types of proteins that are produced (Artlip and Funkhouser, 1995; Close, 1996; Guy, 1990; Gallie, 1993). In peach (*Prunus persica*), several proteins increase during fall and winter, then decline to the point of being nondetectable during the spring and summer (Arora et al., 1992). The presence of these proteins is also correlated with changes in cold tolerance. Arora and Wisniewski (1994) demonstrated that one of the seasonally regulated proteins, PCA60, is a member of the dehydrin family of proteins. Recently, Artlip et al. (1997) have subsequently cloned, sequenced, and characterized the seasonal expression of the gene *ppdhn1* that encodes PCA60.

The dehydrin family of proteins is induced by environmental stresses such as drought or low temperature, which result in cellular dehydration (Close, 1996). Dehydrins are glycine-rich, deficient in tryptophan and cysteine, and remain soluble at 100 °C. In addition, dehydrins are characterized by a lysine-rich amino acid sequence that is almost always present at the carboxy terminus. This amino acid sequence is frequently found repeated throughout the polypeptide and may create an amphipathic helix similar to the molecular chaperone class of proteins (Close, 1996; Dure, 1993). Due to the induction of dehydrins (transcripts and proteins) in response to dehydration and the physical characteristics of the protein, it has been suggested that dehydrins may serve a protective or ameliorative function during environmental stress (Close, 1996). The plant growth regulator abscisic acid (ABA) also induces some dehydrin transcripts and proteins, further supporting the idea that dehydrins may serve a protective function during dehydrative events (Close, 1996).

While much has been learned about dehydrins in herbaceous plants, information on dehydrins in woody plants is limited. It is known that several species of woody plants also display a seasonal pattern in the accumulation of dehydrins similar to peach (Wisniewski et al., 1996). Dehydrin proteins were also present in chilled blueberry (*Vaccinium*, section *Cyanococcus*) floral buds (Muthalif and Rowland, 1994), while chilling and desiccation have been shown to induce accumulation of dehydrin mRNA transcripts in citrus [*Poncirus trifoliata* (L.); *Citrus grandis* (L.) Osb.] leaves (Cai et al., 1995). However, detailed analyses of desiccation and ABA induction of dehydrins in woody plants have not been conducted. Additionally, induction of dehydrins by these factors in different tissue types within the same plant have not been investigated.

The relationship between low water content, ABA, and low temperature is only partially understood. Low temperature can reduce water uptake by roots by decreasing the permeability of the roots and increasing the viscosity of water (Kramer and Kozlowski, 1979). Extracellular freezing events create a water potential gradient that causes water to be drawn out of cells to maintain an osmotic equilibrium (Jones, 1992). Thus, it is not surprising that low temperatures have been reported to induce dehydrins in a variety of herbaceous species (Close, 1996). It has been observed, however, that some dehydrins are primarily induced by low temperature, while others are more strongly induced by desiccation (Palva, 1993). The functional significance of this differential inducibility is unknown. However, it has been suggested that, in plant species with many dehydrin genes [e.g., *Arabidopsis thaliana* (L.) Heyn.], the various dehydrins and their differential inducibility may fulfill specific roles in ameliorating different environmental stresses (Welin et al., 1994).

In contrast to other plant species, peach apparently has only one or two dehydrin genes (Artlip et al., 1997), and evidence for expression of only one of these genes (*ppdhn1*) has been docu-

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mented. The *ppdh1* gene product, PCA60, appears to be the primary dehydrin synthesized in bark during cold acclimation (Artlip et al., 1997). If PCA60 is an important component of cold acclimation (Arora and Wisniewski, 1994, 1996; Artlip et al., 1997), then it may also serve as a general tolerance mechanism for desiccation in peach tissues other than bark. A better understanding of its expression in other tissues and by common dehydrin-inducing agents is important in understanding the regulation of this gene in peach trees and for its potential use as a molecular marker for selecting developing more drought- and cold-tolerant peach genotypes. Additionally, seasonal regulation of dehydrin genes is a unique attribute, and its relationship to induction by acute stresses such as desiccation needs to be understood. The objectives of the present study were to determine if *ppdh1* was inducible by desiccation or ABA in all tissues (i.e., a whole-plant response) and to examine the relationship between expression of *ppdh1*, desiccation, and dehydrin protein (PCA60).

Materials and Methods

TREE GROWTH AND EXPERIMENTAL DESIGN. Forty-five 1-year-old, dormant trees of stem-grafted 'Rio Oso Gem' peach on Lovell rootstock were planted in 12-L pots with equal volumes of Metromix 360 (Grace/Sierra, Milpitas, Calif.). In the spring, the trees were grown in a glasshouse under ambient light and temperature conditions. Trees were watered daily and weekly with Peter's 20-20-20 (w/w; total N-available P-soluble K), using a concentration of 53 mM N. Water deficit or ABA application experiments were initiated in midsummer. All treatments were performed on three replicate trees. At the conclusion of each treatment, leaf, bark, and xylem tissues from each tree were separately harvested and stored. The samples were frozen in liquid N₂ and stored at -80 °C until RNA and proteins were extracted. Xylem tissues required lyophilization (4 d), followed by grinding in a Wiley mill (#60 mesh) before extraction of RNA and proteins.

WATER DEFICIT. Water deficit was imposed on three trees by withholding water until pot weights were 35% of the saturated pot weight. A stem water potential of -1.9 MPa was achieved and maintained for 1 week. Trees then were either harvested or watered daily to pot saturation to alleviate the imposed water deficit. Three rewatered trees were harvested 1 week after daily watering recommenced. Control trees were maintained at field capacity (-0.2 to -0.3 MPa). Water potential measurements on selected shoots were performed predawn with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, Calif.) according to Scholander et al. (1964).

ABA APPLICATION. This treatment consisted of normal watering supplemented with 300 mL of a 100 mM racemic mixture of ABA (Sigma, St. Louis) at 100 mM in 25 mM Tris-HCl, pH 7.6. The ABA solution was applied on alternate days for 1 week, after which time the three trees were harvested. Control trees received buffer without ABA.

PROTEIN ANALYSIS. Bark proteins were extracted in borate buffer [50 mM borate, 50 mM ascorbic acid, 1 mM PMSF (pH 9.0)] at 4 °C as reported by Arora et al. (1992), with immunoblotting as described by Arora and Wisniewski (1994). The blotted proteins were probed with a 1:1000 dilution of polyclonal antibody (provided by Timothy Close) directed against a synthetic peptide of the 15 amino acid consensus region (EKKGIMDKIKEKLPG, one-letter amino acid code) of dehydrins (Close, 1996). This antibody is highly specific, having been used to screen the cDNA library that resulted in the selection of a cDNA clone representing *ppdh1* (Artlip et al., 1997). Analysis of other clones selected from that

library indicated that they were also from *ppdh1* transcripts. This specificity, and the demonstrated homology between PCA60 and the dehydrin transcript (Artlip et al. 1997), allow direct comparisons to be made between the immunodetected protein and transcript levels. Immunoreactive bands were visualized by alkaline phosphatase by using the Proto Blot AP kit (Promega, Madison, Wis.). Each sample used represented an individual tree. Bark proteins from all trees of each sample were obtained, and the protein blot presented here is representative. All lanes were loaded on an equal protein basis (6 µg/lane).

RNA BLOTS. Total RNA was extracted from tissue samples as described by Callahan et al. (1993). Total RNA (10 µg) was separated on 1.4% agarose-formaldehyde gels and transferred to Nytran membranes (S&S, Keene, N.H.) according to the manufacturer's instructions for capillary transfer. The RNA was then UV cross-linked to the membrane (0.3 J·cm⁻²; Bioslink 312, Bios Corp., New Haven, Conn.).

Probe template DNA was derived from a cDNA clone of *ppdh1*, which was isolated from a peach bark cDNA library (Artlip et al., 1997). Membranes were prehybridized, hybridized, and washed according to manufacturer's protocol. In brief, prehybridization (3 h) and hybridization (overnight) were done at 42 °C in 50% formamide/5× Denhardt's reagent/0.1% SDS/100 µg·mL⁻¹ denatured DNA/5× SSPE. The template DNA was radioactively labeled via random priming (Life Technologies, Gaithersburg, Md.). Washes were done with increasing stringency, culminating in a final wash at 65 °C in 0.1× SSPE/0.1% SDS. All operations were performed with probes of the same specific activity. To confirm equal loading of samples, the same blots were stripped and reprobed with an internal fragment of the soybean 18S rRNA gene (courtesy of Carol Bassett).

Results

The water deficit treatment resulted in a predawn stem water potential of -1.9 MPa (Table 1), which has been defined as severe (Hsiao, 1973). The week-long water deficit treatment did not impair the ability of the trees to recover from drought stress, as rewatered trees had water potentials commensurate with the control trees 1 week after watering (Table 1). Applying ABA to well-watered trees did not significantly affect the water potentials of the treated trees compared to the buffer control or normally watered trees.

The probe used in the current study hybridizes to a 1.8-kb transcript that accumulates in peach bark during cold acclimation (Artlip et al., 1997). A transcript of the same size was observed in all tissues in this work suggesting that the same gene is being expressed in these tissues (Fig. 1). Minimal expression of *ppdh1* transcript was seen in bark collected from trees before potting (PP) or before treatments (FL). The 1.8-kb transcript, however, was

Table 1. Mean shoot water potential ± SD of 'Rio Oso Gem' peach trees measured at predawn; n = 3.

Treatment	Water in container medium (% field capacity)	Shoot water potential at harvest (MPa)
Water deficit-Control (FL)	100	-0.34 ± 0.03
Water deficit (SS)	35	-1.92 ± 0.45
Water deficit-Recovery (SR)	35-100	-0.37 ± 0.03
ABA-Control (Con)	100	-0.37 ± 0.20
ABA (100 mM) (ABA)	100	-0.28 ± 0.04

Fig. 1. RNA blot of bark, leaf, and xylem peach tissues subjected to water deficit and subsequent recovery. PP = prepotted, FL = full leaf (well-watered) control, SS = water deficit stress, SR = water deficit recovery. Upper panels show the blot probed with a peach dehydrin cDNA clone and the lower panels show the same blot probed with the internal fragment of a soybean 18S rRNA probe to demonstrate relative amounts of RNA loaded in each sample.

clearly induced in response to water deficit treatment (Fig. 1). The transcript was still present 1 week after recovery from the water deficit but at a lower level. The ABA application also induced transcription in bark tissues, at a higher level than the ABA control (Fig. 2). The likelihood that the differential transcript accumulation is due to isogenes is slight. DNA blot analysis using this probe indicated that, under stringent hybridization and washing conditions used by Artlip et al. (1997) and this research, the probe hybridizes to a single DNA fragment, suggesting a single gene (Artlip et al., 1997).

The pattern of *ppdhn1* accumulation in leaves was generally similar to bark tissue, but with less transcript present (Fig. 1). Conversely, leaves from full-leaf control had levels of *ppdhn1* transcript in greater than in bark. In contrast to bark, however, transcript levels declined to background levels upon water deficit-recovery. ABA application had a minimal effect on transcript levels, being ≈ 2 -fold over the control (Fig. 2).

Transcript abundance in xylem tissues was almost undetectable in the prepotted and full-leaf (well-watered) controls (Fig. 1). As in the bark and leaves, transcript abundance was elevated by water deficit but decreased greatly upon recovery. Transcript abundance was nearly as great as in bark tissues. ABA application elevated transcript levels only slightly above the ABA-control, in which no signal was detectable (Fig. 2).

Protein blotting was done in conjunction with RNA blotting to determine whether or not transcript accumulation was accompanied by accumulation of PCA60 (Fig. 3). In the current study, PCA60 was present in bark and xylem samples taken from prepotted, full-leaf (well watered) control and ABA-control samples (Fig. 3a). Water deficit resulted in a slight to moderate increase in the amount of PCA60. Despite a decrease in transcript level (Fig. 1), the level of PCA60 remained elevated in bark and xylem tissues taken 1 week after resumption of daily watering (Fig. 3). Applying ABA resulted in little to no accumulation of PCA60 above what was present in the ABA-control. The difference in accumulation of dehydrin protein in response to water deficit versus in ABA-treated tissues was similar to the differences observed in transcript levels (Figs. 1 and 2).

PCA60 was not detected in leaves from any of the treatments

(data not shown). In this regard, the protein data for leaves do not agree with the transcript data, which show enhanced accumulation of the *ppdhn1* transcript in response to water deficit (Fig. 1b) and ABA application (Fig. 2).

Discussion

Arora and Wisniewski (1994) and Artlip et al. (1997) demonstrated that a peach dehydrin, PCA60, and its transcript, undergo seasonal accumulation in bark, reaching a peak during the winter. The accumulation kinetics suggested a complex induction mechanism involving photoperiod and low temperatures, but the most significant accumulation of transcript and protein occurred in response to low temperature. The data from this study indicate that *ppdhn1*/PCA60 is also inducible by water deficit and, to some extent, exogenous ABA application. Welin et al. (1994) also demonstrated that two genes (*lti30* and *lti45*) in *Arabidopsis thaliana* that were primarily cold induced were differentially expressed in response to water deficit and ABA. Whereas severe water deficit induced moderate expression of these genes, ABA application resulted in only a slight induction. Our present results for induction of *ppdhn1* are similar in that *pdhn1*, which is induced by low temperature, is more highly induced by water deficit than by ABA. However, while transcript levels declined during the week-long recovery period from water deficit, protein levels remained high. This is in contrast to other studies on dehydrins, where rehydration leads to a cessation of dehydrin transcription within 24 h and some depletion of the protein (Neven et al., 1993; Robertson and Chandler, 1992).

The presence of PCA60 in bark tissues of dormant prepotted trees (Fig. 3) in the absence of transcript agrees with our previous report (Artlip et al., 1997) where we observed that, in early spring, *ppdhn1* transcript levels declined while the amount of protein remained high. No evidence of RNA degradation was observed in either study (data not shown). The disparity between transcript and protein levels indicates that PCA60 has a relatively long half-life. This may be a unique feature of seasonally regulated dehydrins. Artlip et al. (1997) suggested that a pool of stable PCA60 may be necessary to counter potential freezing temperatures and concomitant dehydration, when low temperatures reduce or prevent new transcripts from being produced. If this is true, the apparently long half-life of PCA60 would explain why high levels of the protein were present even after alleviation of the severe water deficit.

Applying ABA resulted in only a slight induction of transcript levels in leaf and xylem tissues and moderate induction in bark tissues (Fig. 2). Increases in PCA60, however, were not evident (Fig.

Fig. 2. RNA blot of bark, leaf and xylem peach tissues treated with ABA. ABA = application of 100 mM ABA, Con = no ABA application. Upper panel shows the blot probed with a peach dehydrin cDNA clone and the lower panel shows the same blot probed with the internal fragment of a soybean 18S rRNA probe to demonstrate relative amounts of RNA loaded in each sample.

Fig. 3. Protein blot of peach tissues probed with a polyclonal dehydrin antibody; (a) bark, (b) xylem, and (c) pre-immune control. PP = prepot, FL = full leaf (well-watered) control, S = water deficit stress, R = water deficit recovery, A = 100 mM ABA application, and AC = no ABA control. L = leaves, B = bark, and X = xylem. Gels were loaded with 6 μ g of protein.

3). These data indicate that, while ABA can induce transcription, the protein is apparently not translated above a basal level or is turned over more rapidly with ABA treatment. PCA60 was not detected in the leaf tissues, suggesting that some other component of the signal transduction pathway for the synthesis of dehydrin may be necessary in leaves. It could be argued that leaves are incapable of synthesizing PCA60 in detectable amounts. Arora et al. (1996) found that peach leaves that had cold acclimated before senescence in the fall contained detectable, although low, amounts of PCA60. Post-transcriptional regulation is a possibility, as the gene for osmotin is also inducible under a wide range of conditions, yet the protein is detected in only a few instances (Kononowicz et al., 1993).

In contrast to many herbaceous species that have several dehydrin genes (Close, 1996), only one dehydrin gene and protein have been documented in peach. The peach dehydrin gene (*ppdhn1*) is unique in that it is the only dehydrin gene that has been shown to be seasonally regulated and does not fall into any of the five distinct types of dehydrins as described by Close (1996). The five types are distinguished by the appearance and position of a tyrosine-containing (Y) segment, a serine-rich tract (S), and lysine-rich (K) segments, which are reduced to a YSK shorthand (Close, 1996). The peach dehydrin gene has a Y_2K_0 arrangement, which represents a hybrid between K_n and Y_nSK_2 types. K_n types are strongly cold induced, while those with an S segment are both cold and desiccation induced (Close, 1996). Whether the hybrid nature of the dehydrin found in peach is characteristic of woody plants in general remains to be determined. Further studies of the promoter region of *ppdhn1* are in progress and will help determine how this gene is regulated developmentally (i.e., seasonally) and by water deficit. The results of this study, in conjunction with previous studies, indicate that, in peach, synthesis of dehydrin is associated with the onset of stress (cold temperature and water deficit), while turnover or disappearance of the protein is not as tightly linked to the alleviation of the inducing stress. Further characterization of the induction and regulation of stress-related genes in peach will be useful in the development of molecular markers for selecting drought- and cold-tolerant genotypes, as well as provide opportunities for the manipulation of these traits via genetic engineering.

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