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Polyphenol Oxidase in Potato[1]

A Multigene Family That Exhibits Differential Expression Patterns

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Polyphenol oxidase (PPO) activity in potato (*Solanum tuberosum*) plants was high in stolons, tubers, roots, and flowers but low in leaves and stems. PPO activity per tuber continued to increase throughout tuber development but was highest on a fresh weight basis in developing tubers. PPO activity was greatest at the tuber exterior, including the skin and cortex tissue 1 to 2 mm beneath the skin. Flowers had high PPO activity throughout development, particularly in the anthers and ovary. Five distinct cDNA clones encoding PPO were isolated from developing tuber RNA. POT32 was the major form expressed in tubers and was found in all parts of the tuber and at all stages of tuber development. It was also expressed in roots but not in photosynthetic tissues. POT33 was expressed in tubers but mainly in the tissue near the skin. POT72 was detected in roots and at low levels in developing tubers. NOR333 was identical with the P2 PPO clone previously isolated from potato leaves (M.D. Hunt, N.T. Eannetta, Y. Haifeng, S.M. Newman, J.C. Steffens [1993] Plant Mol Biol 21: 59–68) and was detected in young leaves and in tissue near the tuber skin but was highly expressed in flowers. The results indicate that PPO is present as a small multigene family in potato and that each gene has a specific temporal and spatial pattern of expression.

PPO is the major cause of enzymic browning in higher plants (Vaughn et al., 1988). PPO catalyzes the conversion of monophenols to *o*-diphenols and *o*-dihydroxyphenols to *o*-quinones. The quinone products can then polymerize and react with amino acid groups of cellular proteins, resulting in black or brown pigment deposits. Such damage causes considerable economic and nutritional loss in the commercial production of fruit and vegetables (Vamos-Vigyazo, 1981). PPO is localized in plastids, and although membrane associated, it is not an integral membrane protein (Vaughn et al., 1988). In vivo, the phenolic substrates of PPO are localized in the vacuole and the browning reaction only occurs as a result of tissue damage leading to a loss of this subcellular compartmentation.

Various physiological roles have been proposed for PPO (Vaughn et al., 1988). Because of the plastidic location of PPO, it was postulated to play a role in the photosynthetic reactions of chloroplasts, but it is now more widely accepted that PPO is probably involved in defense against invading pathogens or insect pests (Mayer, 1987; Steffens et al., 1994). Little information is available about the regulation of PPO gene expression in plants, but a number of PPO genes from different plant species have now been isolated and characterized. All encode peptides of approximately 67 kD that give rise to mature proteins of approximately 60 kD following cleavage of a transit peptide from the N terminus during import into the plastids (Cary et al., 1992; Robinson and Dry, 1992; Shahar et al., 1992; Hunt et al., 1993; Newman et al., 1993; Dry and Robinson, 1994). In some species PPO genes are present as multigene families (Cary et al., 1992; Newman et al., 1993), whereas in others only a single PPO gene has been identified (Dry and Robinson, 1994). All of the PPO genes characterized thus far are nuclear encoded (Cary et al., 1992; Robinson and Dry, 1992; Shahar et al., 1992; Hunt et al., 1993; Newman et al., 1993). PPO contains copper, which is essential for its activity (Delhaize et al., 1985), and the greatest sequence conservation within and between species is around the His residues, which are postulated to bind the prosthetic copper atoms to the protein (Dry and Robinson, 1994).

Most of the PPO genes characterized so far have been isolated from photosynthetic tissues, where the plastids are in the form of chloroplasts, but PPO is also present in nonphotosynthetic tissues. Enzymic browning mediated by PPO is particularly apparent in potato (*Solanum tuberosum*) tubers (Matheis, 1987; Corsini et al., 1992), where the enzyme is localized within amyloplasts of the tuber cells (Czaninski and Catesson, 1974). Two PPO genes previously isolated from potato by Hunt et al. (1993) were found to be expressed in leaves, flowers, roots, and petioles, but no expression was detected in tubers. We now report the isolation and characterization of novel PPO genes from potato tubers. There appear to be a number of different PPO genes in potato plants, each with specific spatial and temporal patterns of expression.

MATERIALS AND METHODS

Plant Material

Potato (*Solanum tuberosum* cv Norchip, cv Saturna, and cv Atlantic) plants and tubers were obtained from commercial fields in South Australia at 3- to 4-week intervals.

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Abbreviations: PPO, polyphenol oxidase; RACE, rapid amplification of cDNA ends.

PPO Activity

Tissues were diced prior to extraction and added to 5 volumes of grinding buffer (25 mM Mes, 10 mM ascorbate, pH 6.0). Enzyme extracts were prepared by homogenizing the tissue with a Polytron blender (Kinematica AG, Littau, Switzerland). Cell debris was removed by filtration through Miracloth (Calbiochem). PPO activity of extracts was measured as the initial rate of oxygen uptake at 25°C in 50 mM sodium phosphate, pH 6.0. The reaction was initiated by the addition of the substrate 4-methyl catechol to a final concentration of 2 mM. One unit of activity was defined as that which catalyzes the consumption of 1 μmol of oxygen per minute under the assay conditions.

Table I. *PPO activity in different potato tissues (cv Atlantic)*

Tissue	PPO Activity
	units g^{-1} fresh wt
Stems	2
Mature leaves	7
Young leaves	17
Roots	144
Stolons	221
Tuber buds	236
Mature tubers	45
Petals	10
Sepals	17
Anthers	81
Ovary, stigma, and style	83

Nucleic Acid Manipulations

Total RNA was extracted from potato tissues according to the method of Logemann et al. (1987). mRNA was isolated using the PolyATract system (Promega). First-strand cDNA was synthesized using avian myeloblastosis virus reverse transcriptase and oligo(dT) primer, and partial PPO cDNAs were amplified by PCR using 3′ RACE (Frohman et al., 1989) with degenerate oligonucleotide primers. PCR products were end filled with Klenow fragment and cloned into pBluescript SK+ (Stratagene). The 5′ ends of PPO cDNAs were obtained by 5′ RACE (Frohman et al., 1989) with nested antisense primers and cloned as described above. DNA sequencing was carried out according to the method of Sanger et al. (1977) with T7 DNA polymerase or *Taq* DNA polymerase. DNA was sequenced on both strands. Northern analysis was carried out as described previously (Robinson and Dry, 1992). When different probes were used, they were of comparable specific activity and blots were exposed to x-ray film for equal times.

Sequence Analysis

DNA and protein sequences were analyzed using the PC/Gene sequence analysis package (Intelligenetics, Inc., Mountain View, CA) and the GCG package (Genetics Computer Group, 1991).

RESULTS

PPO Activity in Potato Tissues

The PPO activity of various potato tissues (cv Atlantic) was determined and the results are shown in Table I. The highest PPO activity was in stolons, tuber buds, and roots, with moderate levels in young leaves and mature tubers and low activity in mature leaves and stems. PPO activity within flowers was high in the reproductive tissues, i.e. the ovary (including the stigma and style) and the anthers, but low in the petals and sepals (Table I). The PPO activity throughout leaf and flower development was further investigated, and the data are shown in Table II. PPO activity in leaves was highest in the youngest stage assayed (<1 cm long) and declined to virtually undetectable levels in fully expanded leaves (5–6 cm long). PPO activity was high throughout flower development with a maximum in large, unopened, not fully developed flowers. Activity in opened flowers decreased by approximately 50% but was still relatively high.

PPO activity in tubers of varying sizes, assumed to reflect tuber age or stage of development, was determined and the results are shown in Figure 1, A and B. The magnitude of PPO activity varied with the cultivar of potato assayed, being 3 to 4 times higher in cv Saturna than in cv Atlantic. In both cultivars PPO activity per tuber accumulated almost linearly throughout tuber development, whereas PPO activity per gram fresh weight of tuber was high in young tubers and declined to a level that remained constant throughout subsequent tuber growth. The distribution of PPO activity within the "peel" and "cortex" fractions is shown in Figure 1, C and D. "Peel" in this instance comprised the outer skin and approximately 1 mm of tuber cortex, which was removed with a common vegetable peeler. The remaining tissue was designated "cortex." On a fresh weight basis, PPO activity of the peel fraction was higher than that of tuber cortex at all stages of tuber development and declined less than the activity in the cortex with increasing tuber size (Fig. 1D). The peel contributed only a small proportion of the weight of the tuber and hence only a small amount of the total PPO activity of the tuber (Fig. 1C). The relative contributions of the peel and cortex fractions to the PPO activity of tubers of cv Saturna were essentially the same as for cv Atlantic (data not shown).

To obtain a finer resolution of the distribution of PPO activity within the tuber, we determined the activity profile along the longitudinal axis of developing tubers. The peel fraction was further subdivided into the skin and the outer cortex (defined as the exterior 1-mm layer of cortex), and the remainder of the cortex was sectioned in 2-mm-thick slices. Each of these fractions was assayed for PPO activity and Figure 2 illustrates that, although the skin contained a significant amount of PPO activity, the highest activity was recorded in the subepidermal or outer cortex layer. PPO activity then decreased to a level that was constant through to the center of the tuber.

Table II. *PPO activity throughout potato leaf and flower development (cv Atlantic)*

Tissue	PPO Activity
	units g^{-1} fresh wt
Leaf stage	
1 (<1 cm)	57
2 (1–2 cm)	33
3 (2–3 cm)	21
4 (5–6 cm)	3
Flower stage	
1 (small buds)	112
2 (medium buds)	128
3 (large buds, not fully developed)	163
4 (large buds, fully developed)	148
5 (open flowers)	77

Isolation and Characterization of PPO Genes from Potato Tubers

Oligonucleotide primers complimentary to conserved regions around the putative copper-binding residues of PPO amino acid sequences from plants (Dry and Robinson, 1994) were synthesized. These primers were used to amplify PPO cDNAs using the 3′ RACE technique (Frohman et al., 1989).

Five distinct cDNA clones, designated POT32, POT33, POT41, POT72, and NOR333, were isolated from young developing tubers (<1 g, cv Norchip) using this approach. The 5′ ends of POT32, POT33, and POT41 were obtained using the 5′ RACE technique with nested antisense primers designed from the original cDNA sequences. POT32 and POT41 are 95% identical at the nucleic acid level. NOR333 was virtually identical (1 bp difference over 1100 bp) with the P2 PPO gene isolated from potato leaves (Hunt et al., 1993). The four clones POT32, POT41, POT33, and POT72 are all 70 to 72% identical with P2. POT32 (and POT41), POT33, and POT72 all share approximately 82% identity with each other.

Analysis of the deduced amino acid sequence of the full-length clones of POT32 and POT33 and the partial sequences of POT41, POT72, and NOR333 revealed considerable similarity to other plant PPO sequences, particularly around the highly conserved copper-binding sites (Steffens et al., 1994), thus confirming their identity as PPO clones.

POT32 is 1958 bp in length and contains an open reading frame of 1791 bp encoding a 597-amino acid peptide of 66.9 kD. POT33 is 1943 bp long, contains an open reading frame of 1797 bp, and encodes a 599-amino acid peptide of 67.5 kD. Amplification of the coding sequences of POT32 and POT33 by PCR from either tuber cDNA or genomic DNA generated fragments identical in size with the cDNA clones, indicating that neither of these genes possesses introns (data not shown). The predicted protein sequences of POT32 and POT33 (Fig. 3) possess putative plastid transit peptide sequences of 89 amino acids. The transit sequences are rich in the hydroxylated amino acids Ser and Thr and the small, hydrophobic amino acids Ala and Val and possess a net positive charge, all characteristics of transit peptides (Keegstra et al., 1989). The transit peptide is known to be cleaved from other PPO proteins to give rise to the mature protein (Sommer et al., 1994). PPO was purified from the cortex of mature potato tubers and the N-terminal amino acid sequence of the 60-kD protein was

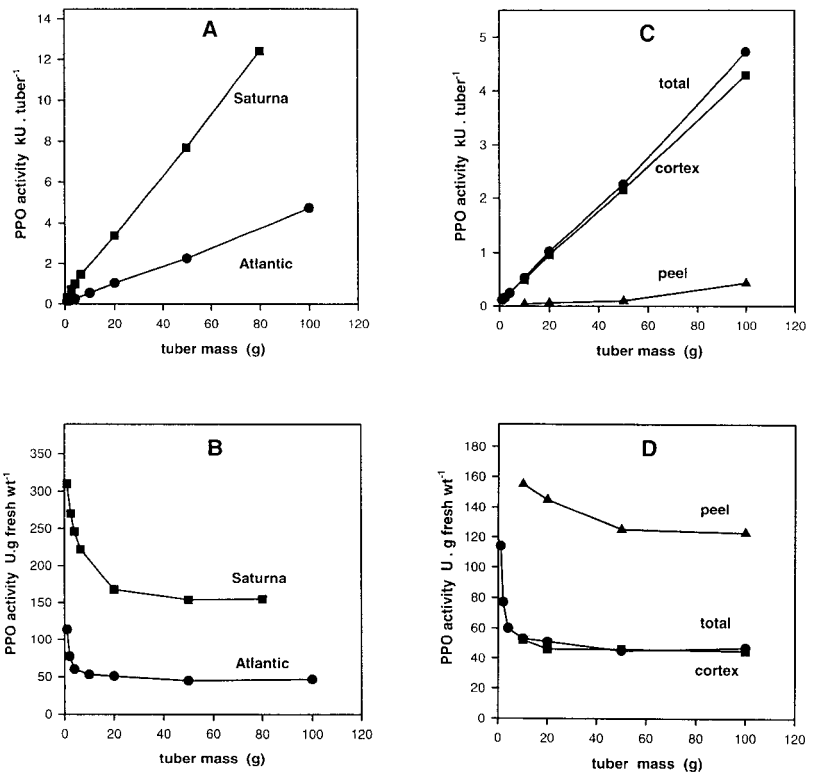

Figure 1. PPO activity in developing potato tubers. A, Accumulation of PPO activity per tuber during growth in cv Atlantic (●) and Saturna (■). B, PPO activity g^{-1} fresh weight (wt) of tuber in cv Atlantic (●) and Saturna (■). C, PPO activity in peel and cortex per tuber of cv Atlantic. ▲, Peel (skin plus approximately 1 mm of cortex); ■, remainder of the tuber; ●, total. D, PPO activity (g^{-1} fresh weight of tuber) in peel (▲), cortex (■), and total tuber (●) of cv Atlantic. kU, Kilounits; U, units.

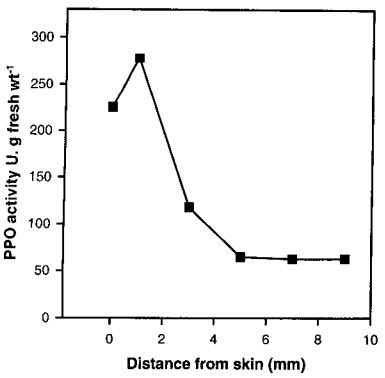

Figure 2. Distribution of PPO activity in a longitudinal section through a tuber. A 70-g tuber (cv Atlantic) was sectioned along a longitudinal axis at 2-mm intervals, and the PPO activity of each section was measured. U, Units; wt, weight.

determined by automated sequencing (Thygesen et al., 1994). This N-terminal sequence from the purified tuber PPO protein (APAPPPDLSS) was identical with the sequence of the mature protein predicted from the clone POT32, confirming that this sequence does indeed encode a potato tuber PPO.

An alignment of the predicted protein sequences of POT32 and POT33 with that of the P2 PPO sequence isolated from potato leaves (Hunt et al., 1993) is shown in Figure 3. The relationship of POT32 and POT33 to each other and to PPOs isolated from other species is shown in Table III. The homology between the PPO protein sequences of potato and tomato (Newman et al., 1993) is very high, and there is an obvious similarity between the species. POT32 and tomato PPO D, POT33 and tomato PPO B, and P2 (=NOR333) and tomato PPO E share greater identity to their interspecific homolog than to the other intraspecific PPOs (Table III).

Spatial Expression of PPO Genes in Potato Tissues

POT32, POT33, POT72, and NOR333 were used to probe RNA isolated from a range of potato tissues, and the results are shown in Figure 4. The unique pattern of expression evident for each of these clones indicates that they are differentially expressed. POT32 was strongly expressed in the inner and outer cortex of tubers and in roots. There was some expression in tuber skin, but POT32 mRNA was not detected in leaves or stems. POT33 mRNA was most highly expressed in the outer cortex of tubers, and POT72 was expressed predominantly in roots with a small amount of message detectable in the inner and outer cortex of tubers.

Probing of potato tissues with the clone NOR333 elicited very strong hybridization to RNA from unopened flower buds, and there was also considerable hybridization to RNA from young leaves but none from mature leaves (Fig. 4). NOR333 also hybridized to RNA isolated from potato tubers (Figs. 4 and 5). However, this hybridization was confined to RNA from the "outer cortex" fraction of the tuber and elicited very poor or no hybridization to RNA from either the skin or inner cortex (Fig. 4). Southern blot experiments with a dilution series of the PPO cDNA clones indicated that there was <5% cross-hybridization between clones at the same stringency used for the northern blots (data not shown). Thus, except for the flower RNA sample, the possibility of hybridization signals resulting from cross-hybridization to other PPO transcripts seems unlikely. The level of NOR333 transcript detected in flowers was so great that faint signals elicited by POT32 and POT33 in flowers (Fig. 4) may be attributed to cross-hybridization to NOR333 transcripts.

Temporal Expression of PPO Genes in Potato Tissues

POT32, POT33, POT72, and NOR333 were used to probe RNA isolated from a developmental series of tubers of cv Saturna (the same as that assayed for PPO activity shown in Fig. 1). RNA was isolated from the whole tuber tissue. The four genes all exhibited a similar trend of expression, being highest in young tubers and lower in older ones; however, the absolute levels of transcript varied considerably (Fig. 5). The greatest hybridization signal was elicited by POT32, and its level was highest in small tubers and lower in larger, older tubers, with transcript still detected in 80 g of tubers. POT32 was the predominant PPO message expressed in these older tubers. NOR333 expression was high in young tubers but decreased markedly in older tubers. The expression of POT33 in tubers was lower than that of either POT32 or NOR333, but it was higher in younger tubers relative to older tubers. POT72 was barely detectable, even in young tubers.

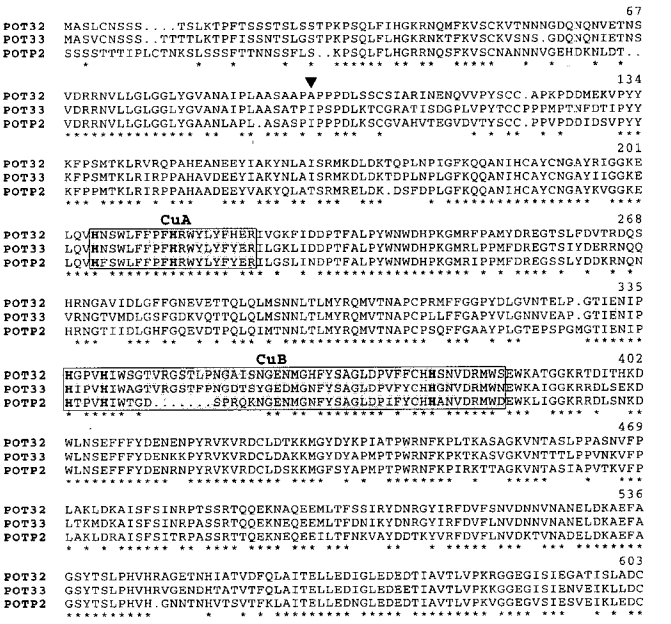

Figure 3. Alignment of potato PPO protein sequences. The deduced PPO protein sequences of POT32 and POT33 were aligned with the P2 PPO protein (Hunt et al., 1993). Identical residues in all three proteins are indicated by asterisks (*); the N terminus of the mature protein is indicated by an arrowhead; the copper-binding regions, CuA and CuB, are boxed and shaded; the His residues predicted to be copper-binding ligands are shown in bold type.

Table III. *Homology of PPO protein sequences*

The percentage of identities of POT32 and POT33 with PPO protein sequences from various plant species were calculated using the "Distances" program of the GCG package. POTP2, Potato leaf (P2, Hunt et al., 1993); Tom B, tomato PPO B; Tom D, tomato PPO D; Tom E, tomato PPO E (Newman et al., 1993); Bean, bean leaf (Cary et al., 1992); Apple, from Boss et al. (1995); Grape, grape berry (Dry and Robinson, 1994). Note that POTP2 is equivalent to NOR333. High interspecific identity of potato and tomato PPOs is indicated in bold type.

	POT32	POT33	POTP2	Tom B	Tom D	Tom E	Bean	Apple	Grape
POT32	100	85.1	79.1	86.1	**93.6**	79.1	54.4	56.3	54.2
POT33		100	80.1	**95.0**	84.4	80.4	53.7	55.7	56.5
POTP2 (=NOR333)			100	80.0	76.2	**94.6**	56.1	56.1	56.3
Tom B				100	85.6	80.1	55.0	56.0	57.1
Tom D					100	76.8	53.6	55.3	53.3
Tom E						100	56.6	56.2	57.8
Bean							100	71.3	63.9
Apple								100	68.5
Grape									100

A more detailed examination of the expression of NOR333 during leaf and flower development is shown in Figure 6. Expression of NOR333 was evident in the youngest leaves (L1), barely detectable in the L2 stage, and not detected in the older leaves. There was strong expression of NOR333 in the four unopened flower stages, F1 through F4. The level of NOR333 mRNA increased with flower age, reaching a maximum at stage F3, declined somewhat in stage F4, and was virtually absent in fully opened flowers, stage F5. Probing of RNA from the reproductive parts of F3-stage flowers indicated that the greatest expression of NOR333 was in the anthers. There was also very high expression in the ovary, considerable expression in petals, and virtually none in sepals.

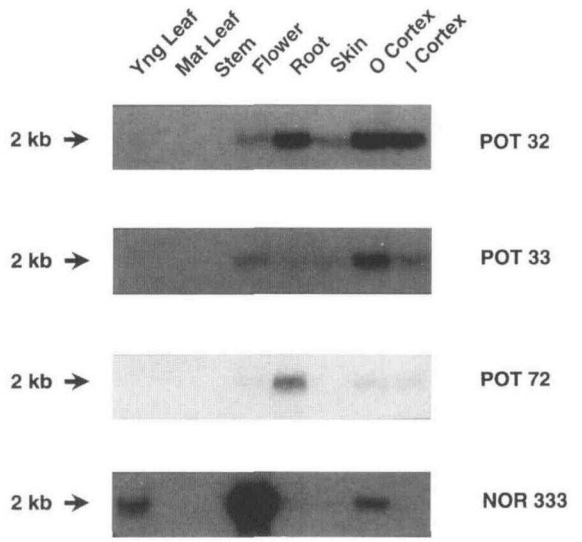

Figure 4. Tissue-specific expression of PPO genes in potato. Total RNA was isolated from potato tissues (cv Atlantic): young leaves (Yng Leaf), mature leaves (Mat Leaf), stems, unopened flower buds (Flower), roots, tuber skin (Skin), the outer cortex of tubers (the tissue 1–2 mm below the skin; O Cortex), and the remainder of the tuber tissue (inner cortex; I Cortex). RNA was extracted from 20-g tubers. RNA (20 μg) from each tissue was probed with POT32, POT33, POT72, or NOR333. The position of the 2-kb RNA marker is indicated beside each panel.

DISCUSSION

We have isolated five distinct cDNA clones encoding PPO from potato tubers, and these clones share considerable homology with other PPO genes, especially those previously isolated from potato leaves (Hunt et al., 1993) and tomato (Shahar et al., 1992; Newman et al., 1993). POT32, POT33, POT41, and POT72 are distinct from the two PPO genes, P1 and P2, previously isolated from potato leaves (Hunt et al., 1993). Somewhat surprisingly, however, NOR333, which was also isolated from tuber cDNA, was found to be identical with the P2 leaf gene.

PPO forms a multigene family of seven distinct genes in tomato (Newman et al., 1993), and Cary et al. (1992) also reported the presence of at least three PPO genes in *Vicia faba*. We now present evidence for a multigene family of at least six PPO genes in potato: the four novel cDNAs reported here and the two genes described by Hunt et al. (1993). The considerable homology of the P1 and P2 and POT32 and POT41 gene pairs of potato and the fact that potato is a tetraploid suggest that these pairs may represent homologs from the different chromosome sets. Consistent with other reports of PPO gene structure (Shahar et al., 1992; Newman et al., 1993; Dry and Robinson, 1994), we found no evidence that either POT32 or POT33 contains introns. This raises the possibility that PPO was originally a chloroplast-encoded gene that was "sequestered" to the nucleus.

The PPO activity in potato tissues was mirrored by PPO gene expression, and this correlation suggests that the control of PPO levels in the potato plant is mediated at the level of transcription. Thus PPO activity was high in developing flowers, developing leaves, and developing tubers and roots (Tables I and II), and the highest levels of PPO mRNA were found in developing flowers and developing tubers and in young leaves (Figs. 4–6). These data are consistent with previous studies in which PPO gene expression has generally been greatest in developing tissues and meristematic regions, with expression declining as development proceeds (Cary et al., 1992; Hunt et al., 1993; Dry and Robinson, 1994; Boss et al., 1995). It is interesting that in the case of tubers PPO activity and gene expression

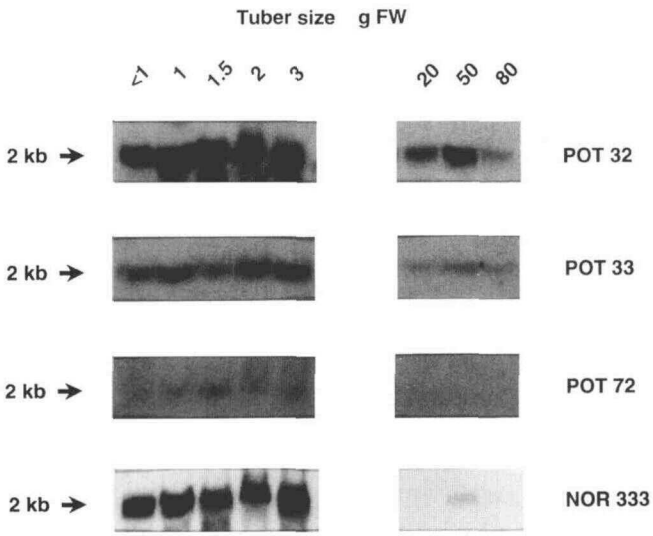

Figure 5. The temporal expression of PPO genes in potato tubers. Total RNA was isolated from whole tubers (cv Atlantic) of varying sizes. Tubers were dug early and later in plant development, and this is indicated by the two separate panels. RNA (20 μg) was probed with POT32, POT33, POT72, or NOR333. The position of the 2-kb RNA marker is indicated beside each panel. FW, Fresh weight.

are maintained beyond early development and throughout tuber development and growth (Figs. 1 and 5).

The control of PPO gene expression in tubers is complex, with the different genes exhibiting distinct patterns of spatial and temporal expression. POT32 and POT33 were expressed predominantly in tubers, with POT32 being the major form detected in all parts of the tuber and throughout tuber development. The identity of the predicted mature N terminus of POT32 with that of PPO purified from tuber cortex provided further verification that POT32 is probably the major mRNA species expressed in tuber cortex. However, given the high degree of homology of POT41 with POT32, we cannot rule out the possibility that POT41 is also highly expressed in these tissues. POT32 was also the major form expressed in roots. POT33 expression was confined to the tuber, where it was most highly expressed in the outer cortex. It was also expressed throughout tuber development but to a much lesser extent than was POT32. The expression of POT72 was barely detectable in tubers and seemed to be almost exclusively expressed in roots but at a much lower level than POT32. POT32, POT33, and POT72 might therefore be considered, if not "tuber genes," at least genes expressed in nonphotosynthetic tissues.

Given that Hunt et al. (1993) detected expression of the P1 gene (95% identical to NOR333) in potato leaves but not in tubers, we were surprised to detect strong expression of NOR333 in tubers. In contrast to POT32 and POT33, NOR333 was expressed only early in tuber development, and its expression was confined to the subepidermal 1 mm, which we termed the outer cortex. We presume that the inner cortex, where there was no expression of NOR333, was analogous to the "mature tuber periderm" probed by Hunt et al. (1993), thus providing an explanation for their failure to detect expression of P1 in tubers.

NOR333 expression in leaves followed the same trend observed for PPO activity: it was highest in very young leaves and declined markedly with leaf age. The expression of NOR333 in young flowers was far in excess of that detected for any of the genes in the tissues tested, perhaps justifying a description as a "floral" rather than a "leaf" gene. Although expression of NOR333 was high in flower buds, it was virtually absent in opened flowers, consistent with the data of Hunt et al. (1993), who detected very low expression of PPO P1 in mature potato flowers. Within the flower the greatest expression was detected in anthers, ovary, and petals, and expression was very low in sepals. This is also consistent with the high PPO activity present in the reproductive tissues. Shahar et al. (1992) investigated the expression of a PPO gene, cP2/A1, in various tomato tissues (cP2/A1 shares considerable homology with NOR333). Expression of cP2/A1 was detected at high levels in the reproductive tissues of flowers 3 to 4 d prior to anthesis and in vegetative meristems. No mRNA expression was detected in the sepals of mature flowers or in tomato fruit. Although it is not possible to make a direct comparison of the developmental stage of the tomato flowers used by Shahar et al. (1992) with the potato flowers assayed in this study, a consistent pattern of PPO expression appears to be evident for the two species. It is possible that the profound reduction of PPO mRNA observed in opened flowers reflects a general shutdown of transcription upon flower opening.

Newman et al. (1993) classified the six PPO genes of tomato into three classes on the basis of restriction fragment length polymorphism mapping. There is extensive homology between individual genes of potato and tomato (>92% identity; Table III) and on the basis of sequence

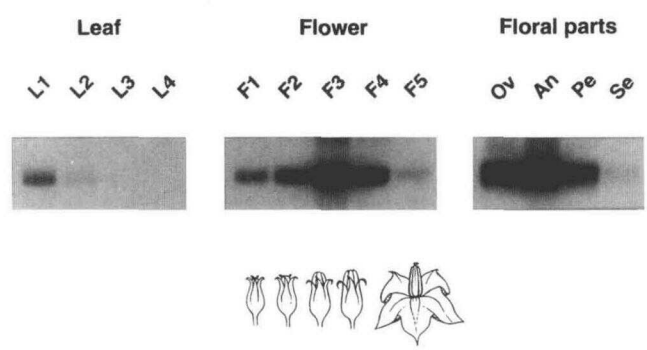

Figure 6. Temporal and spatial expression of the PPO gene NOR333 in potato leaves and flowers. Total RNA was isolated from leaves and flowers of different ages and from floral parts. L1, <1 cm long with yellow-green apices; L2, 1 to 2 cm long; L3, 2 to 3 cm long, green, and still expanding; L4, 5 to 6 cm long, dark green, and fully expanded. F1, Small buds, sepals longer than petals, and petals were green; F2, medium size buds, sepals the same length as petals, and petals still green; F3, buds unopened and still developing, petals longer than sepals, and petals were green-white; F4, buds unopened but fully developed and petals were white; F5, opened flowers. The flower stages are depicted below the panel. Ov, Ovary, stigma, and style; An, anthers; Pe, petals; Se, sepals and pedicel. RNA (20 μg) from each tissue was probed with NOR333. The hybridizing bands were 2 kb in size.

homology each of the genes we have isolated falls into one of these three classes, i.e. class I, POT33 and POT72 (data not shown); class II, POT32 and POT41; and class III, NOR333 (P2) and P1 (Hunt et al., 1993). It is tempting to attribute some functional significance to this conservation of classes of PPO genes between potato and tomato, and an obvious candidate is tissue specificity. Class III genes appear to be expressed in photosynthetic tissues in tomato (Shahar et al., 1992; Newman et al., 1993) and in potato (Hunt et al., 1993; this study). In contrast POT32, POT33, and POT72 are all expressed in nonphotosynthetic tissues of potato. By analogy, therefore, the class I and class II genomic PPO genes of tomato (Newman et al., 1993) would also be predicted to be expressed in nonphotosynthetic tissues, although this remains to be determined. In view of the complex patterns of expression of PPO genes in potato, it would be interesting to isolate and analyze the promoters responsible for regulating the tissue-specific expression of this multigene family, particularly in tubers.

A definite biological function is yet to be attributed to PPO (Vaughn et al., 1988), but it seems likely that it has a role in plant defense against predation (Steffens et al., 1990) or pathogen attack (Mayer, 1987). The maintenance of high PPO activity and gene expression throughout tuber development and the localization of the highest levels at the tuber exterior are consistent with a role in the protection of this storage and propagative organ from predation or infection. High levels in young, developing tissues and in the functional parts of flowers also support such a role. Preliminary experiments indicate that PPO activity and the expression of POT32, POT33, POT72, and NOR333 can be induced to high levels in tubers following wounding (S.P. Robinson and P.W. Thygesen, unpublished results), providing further support for the notion that PPO is an enzyme involved in defense against predation or infection.

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The GenBank accession numbers for the sequences reported in this article are U22921, U22922, and U22923 for POT32, POT33, and POT72, respectively.

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