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Differential Gene Expression in Ripening Banana Fruit[1]

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During banana (*Musa acuminata* L.) fruit ripening ethylene production triggers a developmental cascade that is accompanied by a massive conversion of starch to sugars, an associated burst of respiratory activity, and an increase in protein synthesis. Differential screening of cDNA libraries representing banana pulp at ripening stages 1 and 3 has led to the isolation of 11 nonredundant groups of differentially expressed mRNAs. Identification of these transcripts by partial sequence analysis indicates that two of the mRNAs encode proteins involved in carbohydrate metabolism, whereas others encode proteins thought to be associated with pathogenesis, senescence, or stress responses in plants. Their relative abundance in the pulp and tissue-specific distribution in greenhouse-grown banana plants were determined by northern-blot analyses. The relative abundance of transcripts encoding starch synthase, granule-bound starch synthase, chitinase, lectin, and a type-2 metallothionein decreased in pulp during ripening. Transcripts encoding endochitinase, β-1,3-glucanase, a thaumatin-like protein, ascorbate peroxidase, metallothionein, and a putative senescence-related protein increased early in ripening. The elucidation of the molecular events associated with banana ripening will facilitate a better understanding and control of these processes, and will allow us to attain our long-term goal of producing candidate oral vaccines in transgenic banana plants.

The postharvest physiology of banana (*Musa acuminata* L. cv Grand Nain) is characterized by a green-storage phase followed by a burst in ethylene production that signals the beginning of the climacteric period. Associated with this respiratory climacteric is a massive conversion of starch to sugars in the pulp, during which the activities of the enzymes involved in starch biosynthesis decrease while those involved in starch breakdown and mobilization increase rapidly (Wu et al., 1989; Agravante et al., 1990; Iyare and Ekwukoma, 1992; Cordenunsi and Lajolo, 1995; Hill and ap Rees, 1995a, 1995b). In addition, the rate of respiration rises sharply (Beaudry et al., 1987, 1989). Other changes that occur during ripening include fruit softening, as a result of enzymatic degradation of structural carbohydrates (Agravante et al., 1991; Kojima et al., 1994); a decline in polyphenols that are responsible for the astringency of unripe fruit, catalyzed by polyphenol oxidase and peroxidases (Mendoza et al., 1994); an increase in the activity of alcohol acetyltransferase, the enzyme that catalyzes the synthesis of isoamyl acetate, a major characteristic aroma compound of banana fruit (Harada et al., 1985); and degreening of the peel as a result of chlorophyll breakdown by chlorophyllase (Thomas and Janave, 1992). Stages of banana fruit ripening are scored by PCI numbers, on a scale from 1 (very green) to 7 (yellow flecked with brown) (color preferences chart, Customer Services Department, Chiquita Brands, Inc., Cincinnati, OH). PCI can be correlated with other biochemical and physiological parameters associated with ripening, such as ethylene biosynthesis and respiratory rate, exhibiting peaks at PCI 2 and PCI 4, respectively, in ethylene-treated bananas (Agravante et al., 1991).

Associated with the respiratory climacteric is a large increase in the rate of protein synthesis (Mugugaiyan, 1993), as well as differential protein accumulation (Dominguez-Puigjaner et al., 1992). Polygalacturonase has been identified as a protein that increases in banana pulp during ripening as determined by two-dimensional gel electrophoresis and immunohybridization (Dominguez-Puigjaner et al., 1992). Many of the changes that occur during ripening require de novo protein synthesis (Areas et al., 1988); therefore, a secondary approach to investigate changes that occur during ripening is to isolate transcripts encoding proteins that are associated with the ripening process. Analogous studies of differential gene expression have been employed successfully in other plant species. In kiwifruit differential screening of a fruit cDNA library led to the isolation of three different transcripts, expressed during fruit maturation and ripening, that encode an ATP synthase subunit and two different fruit-specific MT-like proteins (Ledger and Gardner, 1994). Differential screening of an avocado cDNA library generated from cold-stressed fruit yielded clones encoding proteins with homologies to polygalacturonase, endochitinase, Cys proteinase inhibitor, and stress-related proteins (Dopico et al., 1993). Differential screening of a banana pulp library (PCI 1 versus PCI 2) led to the isolation of genes associated with respiratory pathways, cell wall degradation, and stress responses (Medina-Suarez et al., 1997).

Bananas are the intended vector for the production and delivery of candidate oral vaccines for use by children in developing countries (Brown, 1996; Richter et al., 1996). A long-term goal of our research is to identify abundantly expressed transcripts and to utilize promoter elements of these genes to regulate production of foreign proteins in

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Abbreviations: CTAB, hexadecyltrimethyl-ammonium bromide; GBSS, granule-bound starch synthase; MT, metallothionein; PCI, peel color index; PR, pathogenesis-related.

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transgenic banana plants. Differential screening of a tomato cDNA library allowed for the isolation of an abundant, fruit-specific cDNA that accounted for approximately 1% of the mRNA in mature fruit (Pear et al., 1989). If transcript levels and subsequent production of foreign proteins could be attained in banana (i.e. 1% of total protein), it is estimated that a 42-ha banana plantation could satisfy the annual world demand for a single oral immunogen (C. Arntzen, personal communication). Due to our interest in genes encoding abundant ripening-associated transcripts in banana pulp, we employed differential screening and partial sequence analysis of the resulting clones for identification of cDNA isolates. By comparing relative transcript abundance between PCI 1 and PCI 3, we have been able to isolate and identify cDNA clones corresponding to transcripts that are differentially expressed before and after the peak of ethylene biosynthesis in ripening banana fruit, and to determine which of the isolated cDNAs are fruit specific.

MATERIALS AND METHODS

Ethylene-treated and untreated banana (*Musa acuminata* L. cv Grand Nain) fruit were obtained from the Northside Banana Company (Houston, TX). The pulp and peel of fruit representing each of the seven stages of ripening (PCI 1 through PCI 7) were separated and quick-frozen in liquid nitrogen. Tissues from 10 individual fruits were pooled to obtain a uniform, representative sample for each ripening stage and ground to a fine powder under liquid nitrogen in a stainless steel Waring blender. Ground samples were stored at −80°C until utilized.

RNA Isolation

Prewarmed (65°C) RNA extraction buffer (1.4% [w/v] SDS, 2% [w/v] PVP, 0.5 M NaCl, 0.1 M sodium acetate, 0.05 M EDTA, pH 8.0, 0.1% [v/v] β-mercaptoethanol) was added to previously ground samples of pulp from PCI 1 and PCI 3 fruit at a 1:5 tissue:buffer ratio. Samples were homogenized with two or three 30-s pulses from a Polytron tissue homogenizer (Brinkmann) and incubated at 65°C for 15 min. Starch and other cell debris were pelleted by centrifugation at 2,400*g* for 10 min at room temperature, and the supernatant was transferred to a disposable 50-mL polypropylene screw-cap tube. After the addition of 0.2 volume of 5 M potassium acetate, pH 4.8, samples were mixed by inversion and incubated on ice for 30 min. The resulting precipitate was pelleted by centrifugation at 20,200 rpm for 10 min at 4°C in a SW28 rotor (Sorvall). The supernatant was transferred to a disposable polypropylene centrifuge tube, and the high-molecular-weight RNA was precipitated by the addition of lithium chloride to a final concentration of 2.5 M and incubated overnight at 4°C. RNA was pelleted at 4°C for 20 min at 20,000*g* and resuspended in diethyl pyrocarbonate-treated water. The RNA was phenol:chloroform (1:1, v/v)-extracted and ethanol-precipitated.

RNA was isolated from peel, root, corm, and leaf tissues using a CTAB isolation buffer modified from Doyle and Doyle (1987). Root and leaf tissues were ground to a powder in liquid nitrogen using a mortar and pestle. Five grams of frozen powder was added to 10 mL of prewarmed (65°C) CTAB RNA extraction buffer (100 mM Tris-borate, pH 8.2, 1.4 M NaCl, 20 mM EDTA, 2% [w/v] CTAB, and 0.1% [v/v] β-mercaptoethanol). Samples were homogenized with two or three 30-s pulses of a Polytron tissue homogenizer and the homogenate was incubated at 65°C for 1 h. Samples were cooled to room temperature and extracted twice with an equal volume of chloroform, and the phases were separated by centrifugation. Following centrifugation, lithium chloride was added to a final concentration of 2 M, and RNA was allowed to precipitate overnight at 4°C. RNA was pelleted at 4°C for 20 min at 20,000*g*, washed with 70% ethanol, and resuspended in diethyl pyrocarbonate-treated water. The RNA was phenol:chloroform (1:1, v/v)-extracted and ethanol-precipitated.

cDNA Library Construction

Pulp PCI 1 and PCI 3 cDNA libraries were generated using poly(A)$^+$ mRNA prepared from total RNA using a magnetic bead-separation protocol (Dynal), according to the manufacturer's instructions. λ Zap cDNA libraries were generated according to the supplier's protocol (Stratagene).

Differential Screening

Approximately 5×10^4 plaque-forming units from each cDNA library were plated onto Luria broth plates using the appropriate *Escherichia coli* host strain. Duplicate plaque-lifts were generated by placing Nytran nylon filters (Schleicher & Schuell) onto plaque-containing plates for 1 and 4 min for the first and second filters, respectively. Filter-bound DNA was denatured for 2 min in 1.5 M NaCl, 0.5 M NaOH, and neutralized for 4 min in 1.5 M NaCl, 0.5 M Tris (pH 8.0). Filters were rinsed in 0.5 M Tris (pH 8.0), blotted dry, and UV cross-linked (Stratalinker, Stratagene).

Labeled first-strand cDNA probes used in the differential screening were synthesized from 15 μg of total RNA in the presence of 1.5 μM [α-^{32}P]dCTP (3000 mCi/mmol) using an oligo(dT)$_{15}$ primer (Promega) and 15 units of Moloney murine leukemia virus reverse transcriptase, according to the manufacturer's instructions (Promega). The mRNA template was removed by hydrolysis in 100 mM NaOH at 65°C for 30 min. The reaction was neutralized in 100 mM Tris-HCl (pH 8.0), and the labeled first-strand cDNA was ethanol-precipitated in the presence of 20 μg of carrier yeast tRNA.

Filters were prehybridized for 30 min in 1 mM EDTA, 0.25 M phosphate buffer (pH 7.2), 7% (w/v) SDS, and hybridized overnight at 65°C in the same solution containing the denatured probe (1×10^7 cpm/mL). Hybridized filters were washed twice for 30 min each at 65°C in wash solution 1 (1 mM EDTA, 40 mM phosphate buffer, pH 7.2, 5% [w/v] SDS) and three times for 30 min each at 65°C in wash solution 2 (1 mM EDTA, 40 mM phosphate buffer, pH 7.2, 1% [w/v] SDS). The air-dried filters were subjected to autoradiography (X-Omat x-ray film, Kodak) for 72 h at −80°C with an intensifying screen.

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Banana pulp cDNA libraries from PCI 1 and PCI 3 were each probed separately and differentially with labeled cDNA from pulp at PCI 1 and PCI 3. Plaques that demonstrated strong, differential signal intensities between both probes were selected as positives. Positive plaques were then subjected to secondary screening to purify single isolates by utilizing the same probes as in the primary screening. pBluescript phagemids were excised from the isolated plaques according to the manufacturer's recommendations (Stratagene).

Sequence Analyses

Small-scale alkaline lysis plasmid preparations followed by phenol:chloroform extraction and ethanol precipitation (Sambrook et al., 1989) yielded template plasmid DNA suitable for automated sequencing. Plasmid DNA templates were sequenced, using the T3 primer, on an ABI 373A DNA sequencer (Applied Biosystems). Vector and 3′ poly(A) residue sequences were removed from the output sequence. The partial nucleotide sequences obtained were submitted to GenBank, and the accession numbers for the sequences appear in Table I. Edited sequences were loaded into the National Center for Biotechnology Information form for BLAST (9.1) searching on a network server (www.ncbi.nlm.nih.gov), and searches were performed using the default settings of BLASTN (Altschul et al., 1990). For some cDNA clones, no significant homology (defined as a high score above 100) with sequences in the databases was identified using BLASTN. In that event, the default settings of the BLASTX search, an algorithm that translates the nucleic acid sequence in all six frames and searches a nonredundant amino acid database for matches, were used (Gish and States, 1993).

Dot-Blot Hybridization

Comparisons of the relative transcript abundance of the individual cDNA clones between PCI 1, PCI 3, and PCI 5 pulp were made through dot-blot hybridization experiments. Plasmids containing the cDNA inserts were affixed to nylon membrane and hybridized with first-strand cDNA that was generated from PCI 1, PCI 3, or PCI 5 pulp RNA. The equivalent of 10^{11} copies of each plasmid (approximately 0.5 μg of plasmid DNA containing a 1-kb cDNA insert) was heat denatured (95°C for 10 min) and quenched on ice. Using a vacuum dot-blot apparatus (Bio-Rad), target DNA was applied to a nylon membrane (HyBond N^+, Amersham). Membranes were air-dried, UV cross-linked (Stratalinker), and hybridized as described above using 2×10^6 cpm/mL PCI 1, PCI 3, or PCI 5 radiolabeled first-strand cDNA as a probe. Following hybridization, membranes were exposed to a phosphorescent screen (PhosphorImager, Molecular Dynamics, Sunnyvale, CA) and the scanned images were analyzed with the ImageQuant quantitation software.

Northern-Blot Analyses

Total RNA was isolated from banana pulp and peel at PCI 3, and from root, corm, and leaf tissues of greenhouse-grown cv Grand Nain banana plants. Ten micrograms of each of the RNA samples was separated by electrophoresis through formaldehyde-containing agarose gels and transferred to Nytran Plus nylon membrane (Schleicher & Schuell) using a vacuum transfer apparatus (Bio-Rad) according to the manufacturer's recommendations. Equal RNA loading was confirmed by staining the RNA-containing nylon membranes with methylene blue (Sambrook et al., 1989). The RNA blots were hybridized with a cDNA probe representing the largest isolate from each of

Table I. *Genes that are differentially expressed during banana fruit ripening*

The clone designation of a representative of each nonredundant group of cDNA isolates appears in the first column. Indicated in parentheses is the total number of homologous clones isolated. Putative cDNA identities are based on sequence homology. High scores, an indication of the significance of the homology, were obtained using either the BLASTN or BLASTX search algorithms. Changes in relative transcript abundance in pulp between PCI 1 and PCI 3 are indicated as up or down based on dot-blot hybridizations. Transcript sizes were estimated from northern-blot analyses of pulp total RNA.

Representative Clone	Homology to	High Score [P(N)][a]	GenBank Accession No.	PCI 1 to PCI 3	Transcript Size
					kb
pBAN 3-14 (2)	Sweet potato starch synthase	198 [6.8e-6]	AF001526	Down	2.2
pBAN 3-1 (4)	Cassava GBSS	1121 [6.5e-95]	AF001531	Down	2.2
pBAN 3-30 (10)	Winged bean chitinase	300 [7.9e-31]	AF001522	Down	1.2
pBAN 3-24 (2)	Rice endochitinase	773 [3.4e-93]	AF001524	Up	1.2
pBAN 1-3 (2)	Soybean β-1,3-glucanase	524 [3.4e-33]	AF001523	Up	1.3
pBAN 3-28 (2)	Katemfe fruit thaumatin	635 [3.0e-125]	AF001528	Up	1.0
pBAN 3-25 (1)	Rice ascorbate peroxidase	1294 [4.0e-110]	AF001529	Up	1.1
pBAN 3-6 (5)	Kiwifruit MT	218 [1.7e-11]	U49044	Up	0.5
pBAN 3-23 (6)	Castor bean MT type 2[b]	518 [2.4e-33]	AF001525	Down	0.6
pBAN 3-32 (3)	Jack fruit lectin (α subunit)[c]	177 [2.0e-19]	AF001527	Down	0.8
pBAN 3-46 (1)	Asparagus senescence-related gene[c]	167 [3.1e-16]	AF001530	Up	1.0

[a] Probability of homology occurring by chance (see Altschul et al., 1990). [b] Type-2 classification determined by deduced amino acid sequence alignment. [c] BLASTX algorithm used for homology search.

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the 11 nonredundant groups of clones. DNA probes were synthesized using the Rad-Prime DNA Labeling System (GIBCO-BRL), and hybridized as described above. Transcript size was estimated by comparing the position of the hybridizing band to RNA molecular weight standards (Promega).

RESULTS

Differential screening of approximately 10^5 plaques with labeled pulp cDNAs resulted in the identification of approximately 100 plaques with a signal intensity sufficient to be detected by autoradiography after a 72-h exposure to x-ray film. It was apparent from the signal intensities observed between differentially hybridized plaque lifts that the relative abundance of a number of transcripts changed between PCI 1 and PCI 3. Of approximately 80 differentially expressed positives that were selected from the initial screen, 38 cDNA clones were isolated from the banana pulp libraries after two additional rounds of screening.

Sequence alignment and homology searches indicate that 11 nonredundant groups of cDNAs were identified (Table I). Using sequence homology, BLAST searches were able to assign, with high scores between 167 and 1294, a putative identity for all clones. Amino acid sequence homology searches using the BLASTX algorithm were necessary to assign an identity to the clones encoding the putative lectin and senescence-related protein. According to the results of the sequence homology searches, all of the banana sequences are more similar to other plant genes than to genes from other organisms. There were many redundant isolates, especially of the smaller cDNAs such as those encoding the different MTs. For example, 10 homologous clones encoding a putative chitinase, an especially abundant protein in banana pulp (S.K. Clendennen, unpublished data), were isolated (represented by pBAN 3-30; see Table I).

Relative ripening-related transcript abundance in banana pulp was estimated by hybridizing isotopically labeled first-strand cDNA to an excess of cloned cDNA that was previously dot-blotted onto nylon membrane. This technique also allowed for the confirmation of differential expression of these transcripts in pulp between PCI 1 and PCI 3, and at a later stage of ripening, PCI 5 (Fig. 1). Relative transcript abundance of starch synthase, GBSS, lectin, chitinase, and a type-2 MT decreased in pulp between PCI 1 and PCI 3, and continued to decrease through PCI 5. There was a peak in the abundance of several of the transcripts in PCI 3 pulp, including endochitinase, glucanase, thaumatin, ascorbate peroxidase, and MT. The differential expression of these banana transcripts before and after the peak in ethylene biosynthesis indicates that the transcripts that increase in abundance between PCI 1 and PCI 3 may be responding to ethylene. The differential expression of the 11 different groups of cDNAs in banana pulp between ripening stages PCI 1 and PCI 3 was confirmed by northern-blot analyses (data not shown). Results from the dot-blot hybridization were also used to estimate relative abundance of each class of cDNA in the pulp of ripening banana fruit, with thaumatin and β-1,3-glucanase being the first and second most abundant transcripts, respectively (Fig. 1).

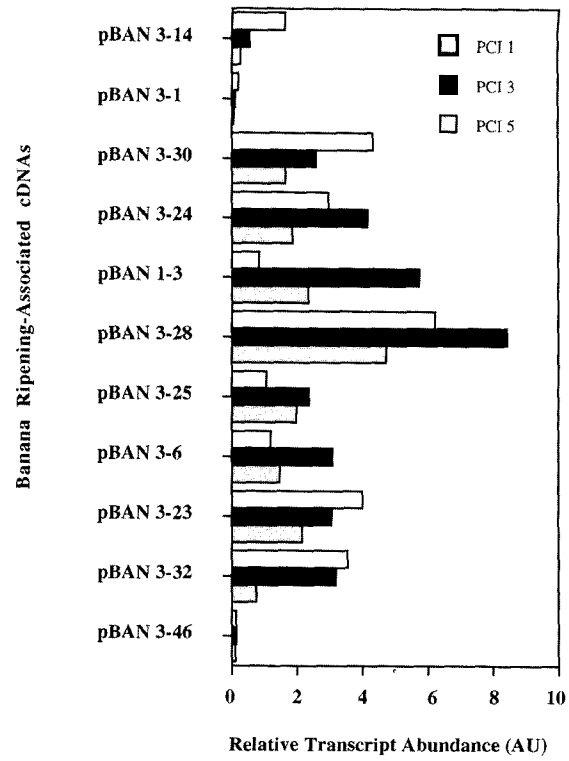

Figure 1. Relative abundance of ripening-associated transcripts in ripening banana pulp. Plasmids containing the indicated cDNA were affixed to nylon membrane and hybridized with radiolabeled first-strand cDNA synthesized from pulp RNA at PCI 1, PCI 3, and PCI 5. Relative transcript abundance was estimated as the activity that hybridized to the target DNA, and is expressed in arbitrary units. The putative identities of the cDNA clones (pBAN 3-14, starch synthase; pBAN 3-1, GBSS; pBAN 3-30, chitinase; pBAN 3-24, endochitinase; pBAN 1-3, β-1,3-glucanase; pBAN 3-28, thaumatin-like protein; pBAN 3-25, ascorbate peroxidase; pBAN 3-6, MT; pBAN 3-23, type-2 MT; pBAN 3-32, lectin subunit; and pBAN 3-46, putative senescence-related protein) are also listed in Table I.

Although these cDNAs are relatively abundant in the pulp of banana fruit, their patterns of expression are not limited to these tissues. Northern-blot analyses indicate that starch synthase, GBSS, and chitinase transcripts were abundant in pulp and corm tissues, and present in peel (Fig. 2). Expression of the endochitinase, thaumatin-like protein, and β-1,3-glucanase transcripts was limited to the pulp and peel of the fruit. Both classes of MT transcripts were expressed in all tissues analyzed, but were most abundant in the pulp and peel. In comparison, MT was more abundant in leaves than type-2 MT, whereas the converse was observed in the corm. Lectin transcripts were most abundant in pulp and root tissues, whereas the ascorbate peroxidase and senescence-related protein transcripts were ubiquitously expressed.

DISCUSSION

Transcripts corresponding to two different enzymes involved in starch synthesis were identified through differ-

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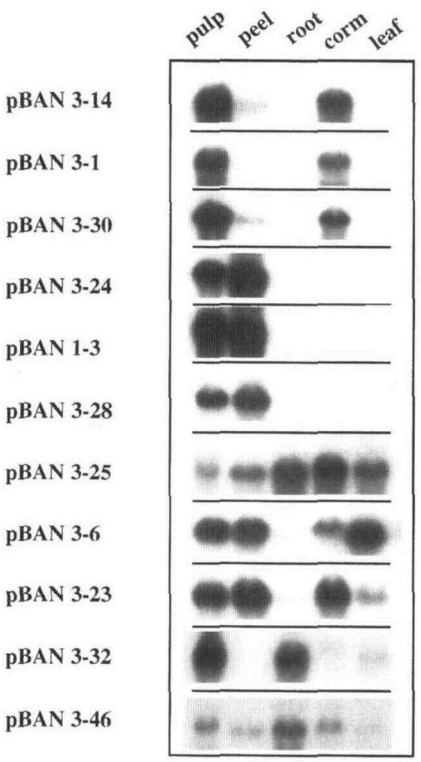

Figure 2. Tissue distribution of ripening-associated transcripts from banana pulp was determined by northern-blot analysis of total RNA from pulp and peel (both at PCI 3), root, corm, and leaf tissues. Blots were hybridized with cDNA probes representing each of the 11 classes of differentially expressed transcripts. The clone designation (pBAN 3-14, starch synthase; pBAN 3-1, GBSS; pBAN 3-30, chitinase; pBAN 3-24, endochitinase; pBAN 1-3, β-1,3-glucanase; pBAN 3-28, thaumatin-like protein; pBAN 3-25, ascorbate peroxidase; pBAN 3-6, MT; pBAN 3-23, type-2 MT; pBAN 3-32, lectin subunit; and pBAN 3-46, putative senescence-related protein) is indicated at the left of each panel.

ential screening. Starch synthase and GBSS belong to the suite of biosynthetic enzymes responsible for the accumulation of starch reserves in pulp amyloplasts during fruit filling. In ripening banana fruit, transcript levels of starch synthase and GBSS appear to decrease, consistent with the observed decrease in the activity of these enzymes (Cordenunsi and Lajolo, 1995).

Chitinase, endochitinase, β-1,3-glucanase, and a thaumatin-like protein were represented by abundant transcripts in ripening banana fruit. These proteins have been associated with a general stress response in plants that is characterized by the expression of a group of genes encoding PR proteins (Ohashi and Ohshima, 1992; Stintzi et al., 1993). Pathogen challenge, stress, wounding, and exposure to ethylene have all been shown to induce expression of PR proteins in plants (LeGrand et al., 1987; Mauch et al., 1988; Kirsch et al., 1993; Dalisay and Kuc, 1995; Shinshi et al., 1995). Studies of PR proteins in fruit include the investigation of wound responses in tomato, which results in the increase of PR proteins (Mehta et al., 1991). Several cDNA clones that encode proteins with homology to endochitinase and a number of other stress-related proteins have been isolated from cold-stressed avocado (*Persea gratissima* L.) fruit (Dopico et al., 1993). Data from the present study suggest an association between the expression of PR proteins and fruit ripening. A thaumatin-like protein and its corresponding mRNA were also found to accumulate to high levels in ripening cherries (*Prunus avium* L.), a nonclimacteric fruit (Fils-Lycaon et al., 1996). In cherry it was considered unlikely that the response was due to infection by a pathogen, but instead suggested at least some similarity between PR responses and the ripening process.

Ascorbate peroxidase is a hydrogen peroxide-scavenging enzyme that is specific to plants and algae and acts to minimize oxidative damage from by-products of photosynthesis and respiration. The ascorbate-dependent hydrogen peroxide-scavenging pathway (for review, see Asada, 1992) is found in chloroplasts and plant mitochondria, and ascorbate peroxidase activity has been correlated with high metabolic rates (Klapheck et al., 1990; Kevers et al., 1992; Dalton et al., 1993). Ascorbate peroxidase transcript abundance increases in banana pulp during ripening, coincident with the increased respiratory activity associated with the climacteric period.

The role of many of the putative translation products represented by the identified banana cDNA clones is not well understood in plants. Transcripts encoding a lectin and two classes of MT were abundant in banana pulp during ripening. In legumes lectins play a role in signaling between the plant host and symbiotic nitrogen-fixing bacteria that colonize root nodules (Sharon and Lis, 1989; Kennedy et al., 1995). It has recently been suggested that lectins may play a role in the sequestration of storage proteins into vacuoles in vegetative tissues (Spilatro et al., 1996). Banana MT clones were tentatively classified as either fruit-type or type-2 MTs based on comparisons of their putative translation products with other MT amino acid sequences. MTs are small Cys-rich polypeptides found in animals, fungi, and plants. There is little evidence for their function in plants, but because they bind to divalent metal ions such as copper, cadmium, and zinc, they may be involved in heavy metal tolerance and play a role in the metal ion flux during normal cell function (Robinson et al., 1993). Clones encoding two different MT proteins were also isolated from developing kiwifruit (*Actinidia deliciosa* var *deliciosa*) by differential screening, although their function in fruit development and ripening is unknown (Ledger and Gardner, 1994). It is also possible that translation products associated with these cDNA clones have no direct function in ripening, but are postharvest senescence-related genes, as suggested by the isolation of a cDNA clone with homology to a putative senescence-related gene from asparagus.

We did not isolate transcripts encoding the proteins associated with some of the obvious changes that occur during fruit ripening in banana. For example, the enzyme activities of phosphofructokinase, Suc-P synthase, starch phosphorylase, amylase, and invertase have been shown to increase in banana pulp during ripening, reaching their highest levels just before or during the respiratory peak (Wu et al., 1989; Agravante et al., 1990; Iyare and Ekundayo, 1992; Cordenunsi and Lajolo, 1995; Hill and ap

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Rees, 1995a, 1995b). However, no cDNA clones corresponding to enzymes with obvious involvement in the respiratory pathway or in starch degradation were isolated, indicating that these transcripts may not be abundant in pulp at the stages of ripening that we examined. It is also possible that the increases in enzyme activity observed during ripening may be due to translational or posttranslational mechanisms, such as phosphorylation or substrate availability (Beaudry et al., 1989; Wu et al., 1989; Law and Plaxton, 1995).

Many of the physiological changes that occur during banana fruit ripening are in response to ethylene produced in the pulp (Dominguez and Vendrell, 1993; Burdon et al., 1994). In addition, ethylene also serves as a signal for other physiological changes, including senescence. The cDNA clones identified in this study were isolated by differential screening at stages of fruit ripening, corresponding to periods before and after the peak in ethylene biosynthesis (Agravante et al., 1991). Therefore, it is likely that some of the transcripts that increase in abundance between those stages of ripening may be regulated by ethylene, even if they do not have a direct role in the ripening process. The role of ethylene in the regulation of PR proteins (glucanase, chitinase, endochitinase, and thaumatin) has been well documented. Ethylene is also believed to influence expression of ascorbate peroxidase (Mehlhorn, 1990) and MT (Coupe et al., 1995).

Identifying ripening-related transcripts that are abundant in banana pulp will enable a more in-depth understanding of the physiological events that occur during ripening. It is now possible to use the isolated cDNA clones in experiments designed to investigate patterns of tissue- and developmental stage-specific gene expression as a result of physiological and environmental changes, or to determine which of these genes respond in a positive or negative fashion to ethylene. With information about relative abundance and spatial- and temporal-expression patterns, the potential roles of the translation products can be examined more rigorously. In addition, genomic clones of some of the more abundant transcripts have been isolated for promoter characterization. These regulatory elements will allow for spatial and temporal control of foreign protein expression in bananas and will facilitate the genetic manipulation of fruit ripening, alteration of the nutritional value, or engineering of pest resistance in banana cultivars. As previously stated, this research was directed at gaining a better understanding of the molecular events associated with banana fruit ripening. It is our aim to build upon this understanding to develop strategies for the expression of candidate oral vaccines in fruit of transgenic banana plants.

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