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A cDNA Clone Highly Expressed in Ripe Banana Fruit Shows Homology to Pectate Lyases[1]

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A cDNA clone (*BanI7*), encoding a protein homologous to pectate lyase, has been isolated from a cDNA library from climacteric banana fruit by means of differential screening. Northern analysis showed that *BanI7* mRNA is first detected in early climacteric fruit, reaches a steady-state maximum at the climacteric peak, and declines thereafter in overripe fruit. Accumulation of the *BanI7* transcript can be induced in green banana fruit by exogenous application of ethylene. This demonstrates that expression of this gene is under hormonal control, its induction being regulated by the rapid increase in ethylene production at the onset of ripening. The deduced amino acid sequence derived from the *BanI7* cDNA shares significant identity with pectate lyases from pollen and plant pathogenic bacteria of the genus *Erwinia*. Similarity to bacterial pectate lyases that were proven to break down the pectic substances of the plant cell wall suggest that *BanI7* might play a role in the loss of mesocarp firmness during fruit ripening.

Many physiological and biochemical changes accompany the initiation of fruit ripening. In climacteric fruit, in addition to alterations in pigment biosynthesis and the production of flavor compounds, a major increase in the synthesis of ethylene and an increase in respiration also occur. Ripening is also accompanied by a solubilization of the cell wall, which leads to fruit softening. Changes in texture during ripening are one of the major quality traits that determine fruit shelf life. Although these textural changes occur gradually in many fruits, in others, such as banana and strawberry, they take place very rapidly and often dictate the very short postharvest periods characteristic of these fruits. Tissue softening is also associated with an enhancement in pathogen susceptibility and frequently results in fungal growth at the later stages of ripening.

Fruit softening appears to be mainly associated with changes in the pectin fraction of the cell wall (Huber, 1983). Textural changes are accompanied by the cleavage of methyl ester groups from the pectin backbone (Carpita and Gibeaut, 1993), a large increase in pectin solubilization, and a reduction in pectin chain length (Seymour et al., 1987; DellaPenna et al., 1990; Smith et al., 1990). There is also a considerable loss of neutral sugar residues from the pectic fractions of the cell wall (Gross and Wallner, 1979).

These ultrastructural and chemical changes are temporarily correlated with de novo synthesis of several cell wall hydrolases, mainly PG and PME (Tucker and Grierson, 1987; Fischer and Bennett, 1991). While PME acts to remove the methyl group from the C-6 position of the galacturonic acid residues that make up the pectin backbone, PG hydrolyzes the α(1–4) link between adjacent demethylated galacturonic acid residues. These two enzymes thereby act synergistically, with PME generating sites for PG action (Pressey and Avants, 1982; Koch and Nevins, 1989).

It has often been suggested that PG is primarily responsible for pectin degradation and fruit softening (Hobson, 1965; Themmen, et al., 1982; Brady et al., 1983). However, PG-mediated pectin degradation alone cannot account for the significant changes in fruit texture that accompany ripening. In transgenic tomato plants antisense inhibition of PG activity to as little as 0.5% of wild-type levels does not prevent fruit softening (Smith et al., 1990; Schuch et al., 1991). Conversely, expression of a PG transgene in the tomato ripening mutant *rin* results in pectin solubilization and depolymerization at near wild-type levels, but fruit softening still fails to take place (Giovannoni et al., 1989; DellaPenna et al., 1990).

Similar results were obtained in tomato fruit in which PME was down-regulated using antisense RNA technology (Tieman et al., 1992; Hall et al., 1993). These results indicate that in addition to PG and PME, other hydrolytic enzymes are likely to be involved in the textural changes associated with ripening.

In the present study we report on the structure and fruit-specific expression of a banana cDNA clone with homology to pectate lyase. By differential screening from a cDNA library of ripe banana fruit, the cDNA clone *BanI7* was isolated and shown to be specifically expressed in ripe fruit. *BanI7* shows significant protein-sequence similarity to the pectate lyases of style and pollen of various plant species (Wing et al., 1989; Budelier et al., 1990; Rafnar et al., 1991) and to extracellular pectate lyases of plant pathogenic bacteria of the genus *Erwinia*.

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Abbreviations: PG, polygalacturonase; PME, pectin methylesterase.

MATERIALS AND METHODS

Green banana fruits (*Musa acuminata* cv Dwarf Cavendish) were obtained from Tenerife (Canary Islands, Spain). Fruits were removed from the bunch, treated with fungicide solution (0.1% benlate, 0.3% dithane, w/v), and placed in ventilated jars at 22°C. The various ripening stages were selected by measuring the production of CO_2 and the ethylene evolution of single fruits. Stage E1 fruits are green fruits, before the increase of ethylene. Stage E2 fruits are early climacteric fruits at the initiation of the ethylene peak. Stage E3 fruits are climacteric fruits at the respiratory peak; at this stage ethylene has started to decrease and the fruit begins to turn yellow. Stage E4 fruits are ripe, yellow fruits.

For ethylene induction, green fruits were placed in 2-L jars and flushed with a stream of water-saturated air in which C_2H_4 was added to a final concentration of 100 parts per million. Fruits were kept under this ethylene atmosphere for 24 h at 22°C. Control fruits were treated with water-saturated air for 24 h at 22°C.

RNA Isolation and Gel-Blot Analysis

Total RNA was extracted from fruits following a protocol based on the method described by Varadarajan and Prakash (1991). Crude RNA preparations were purified through a Qiagen (Chatsworth, CA) column to remove polysaccharide contaminants. One gram of frozen pulp tissue was ground to powder in liquid nitrogen, homogenized in 10 mL of 0.1 M Tris-HCl, pH 7.4, 50 mM EDTA, 0.5 M NaCl, 2% (w/v) SDS, and 1% (v/v) 2-mercaptoethanol, and incubated for 15 min at 65°C. To this homogenate 3.3 mL of 5 M potassium acetate was added, and the mixture was incubated at −20°C for 10 min. Cellular debris were then removed by 50 min of centrifugation at 10,000*g*, and the RNA was precipitated from the supernatant by adding 0.7 volume of cold isopropanol. The mixture was incubated for 1 h at −20°C, and the RNA was recovered by centrifugation at 10,000*g*. The precipitate was resuspended in a small volume of Tris-EDTA buffer and purified through a Qiagen column following the manufacturer's instructions.

RNA (15 μg of total RNA per lane) was fractionated on a 1.5% agarose/formaldehyde denaturing gel and transferred to Hybond (Amersham) blotting membranes (Ausubel et al., 1992). The DNA probes were radiolabeled using a random-primed DNA labeling kit (Boehringer Mannheim). Hybridization conditions were as described by Amasino (1986). Filters were washed three times for 30 min each with 3× SSC, 0.5% SDS at 65°C. Equal loading on the gel was assessed by including ethidium bromide in the loading buffer. Complete transfer to membrane was verified by UV examination of the membrane after blotting. Poly(A^+) RNA was purified using an mRNA isolation system (PolyATract, Promega), according to the instructions provided by the manufacturer.

cDNA Library Construction and Screening

A cDNA library (0.8×10^6 primary transformants) was constructed from ripe banana pulp poly(A^+) RNA using a cDNA synthesis kit (λ-ZAP, Stratagene). Standard methods described by the manufacturer were used for cDNA synthesis and cloning.

Ripe-specific cDNA clones were isolated by differential plaque hybridization using radiolabeled, single-stranded cDNA probes prepared from poly(A^+) RNAs isolated from unripe and ripe banana fruits, respectively. Plaques hybridizing preferentially to the ripe probe were purified to homogeneity, and the corresponding pBluescript plasmids were in vivo-excised as described in the kit manual.

DNA Isolation and Southern Analysis

Genomic DNA was prepared from nuclei isolated from leaves as described by Prat et al. (1989). Nuclei were collected by centrifugation at 1000*g* for 5 min, DNA was extracted and purified through a gradient of CsCl that contained 1% sarcosyl. Total DNA (10 μg) was digested with appropriate restriction endonucleases, separated by electrophoresis in a 0.8% agarose gel, denatured, and transferred to a nylon membrane. Hybridization and washing conditions were as for northern analysis.

DNA Sequence Analysis

Sequence analysis was performed by the dideoxy chain termination method using the T3 and T7 primers of the pBluescript polylinker and specific primers within the cDNA insert. An automated laser-fluorescent DNA sequencer (Pharmacia) was used to generate the sequence data. Sequence processing and database searches were carried out using the Genetics Computer Group (Madison, WI) programs.

RESULTS

Isolation of Banana Fruit Ripening-Specific cDNA Clones

To isolate genes that showed an elevated expression during fruit ripening, a cDNA library was established from poly(A^+) RNA extracted from ripe banana fruit. Using differential hybridization to cDNA probes from green and ripe fruit, several clones were identified that showed a higher signal with the ripe probe. One of these clones, designated *Ban*I7, which corresponds to an mRNA that is differentially expressed and very abundant in ripe banana fruit, was chosen for further characterization.

*Ban*I7 Gene Expression during Fruit Ripening

To determine the pattern of accumulation of the *Ban*I7 transcript during fruit development, an RNA gel blot was prepared from RNAs isolated from banana fruits at different developmental stages and hybridized with the *Ban*I7 probe. Northern analysis revealed an mRNA of 1.5 kb, indicating that the original clone corresponded to a nearly full-length copy of the transcript. Figure 1 shows that the levels of *Ban*I7 mRNA increase dramatically at the onset of ripening. Maximal levels of the transcript are detected at the respiratory climacteric peak, when the fruit begins to turn yellow (Fig. 1, lane E3). *Ban*I7 mRNA levels declined in the postclimacteric peak (yellow fruit; Fig. 1, lane E4),

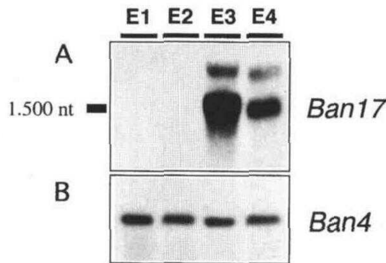

Figure 1. *BanI7* mRNA expression during banana fruit ripening. Fifteen micrograms of total RNA isolated from banana pulp at four stages of ripening was loaded in each lane. Lane E1, Green or preclimacteric fruit; lane E2, early climacteric; lane E3, maximum of respiratory climacteric; lane E4, postclimacteric or ripe fruit. The ripening stage of the fruit was assesed by monitoring the rate of ethylene and CO_2 production. The filter was probed with the *BanI7* cDNA fragment (A) and subsequently hybridized with the *Ban4* cDNA probe (B) that corresponds to an extensin or Pro-rich protein transcript constitutively expressed in the fruit. Blots were exposed for 12 h and identical specific activities were used in both hybridizations.

with a weak signal visible in RNA from overripe fruit (data not shown). No signal was observed in RNA from green or early climacteric fruit (Fig. 1, lanes E1 and E2, respectively). After a longer film exposure *BanI7* mRNA could be detected at the E2 stage of ripening, but still no signal was observed in green fruit, even after prolonged exposures. Likewise, no detectable expression of *BanI7* mRNA was found in leaves (data not shown).

The extensin clone *Ban4*, which has constitutive levels of expression in banana fruit (E. Domínguez-Puigjaner, unpublished data), was used to estimate the relative amounts of RNA per lane (Fig. 1B).

BanI7 Shows Similarity to Pectate Lyases from *Erwinia* spp. and Pollen

The complete nucleotide and predicted amino acid sequences of clone *BanI7* are shown in Figure 2. The cDNA insert is 1462 bp long and encodes a protein of 398 amino acid residues, with a molecular mass of 44 kD. The initial Met is missing from the cDNA insert, and by comparison with the predicted amino acid sequence of related proteins it is concluded that a few nucleotides from the transcript are lacking. Subsequent screening of the cDNA library using a radiolabeled 5′ fragment from the *BanI7* cDNA, however, led to the isolation of clones of the same length or shorter than the original cDNA. Similarly, when rapid amplification of cDNA ends-PCR was used to amplify the 5′ region of the transcript, only partial cDNA sequences were obtained, which were the same length as those present in the *BanI7* clone. These results suggest that some sort of secondary structure is formed at the 5′ RNA leader, which hinders this region from being copied by the reverse-transcriptase enzyme.

The *BanI7* polypeptide includes a highly hydrophobic region at the amino terminus that has the characteristics of a signal peptide for cotranslational translocation into the ER. A putative cleavage site for a signal peptidase (von Heijne, 1986) is located between Ala-17 and Ala-18. A potential N-linked glycosylation site (Asn-X-Ser/Thr) is present in the mature protein (Fig. 2).

Comparison of the predicted amino acid sequence for *BanI7* with sequences in the EMBL database revealed significant homology to a putative pectate lyase from stylar transmitting tissue from tomato (Budelier et al., 1990) and to pectate lyase homologs from maize, tobacco, tomato, and ragweed pollen (Wing et al., 1989; Rafnar et al., 1991; Rogers et al., 1992; Turcich et al., 1993). At the amino acid level *BanI7* shows 74.8% identity to the tomato stylar pectate lyase and 59.7%, 55.2%, 53.4%, and 46.5% identity to

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   1  GCAGCCTTCACTGCCCCCATCTACTCTTCTCGTGCACCCTTAACGTCGGCAGCTGTCCGC
   1  A  A  F  T  A  P  I  Y  S  S  R  A  P  L  T  S  A  A  V  R     20
MSTL  F  F  T  F  S  L  L  L  L  .  A  P  LL V  I  S ▲S  I  Q     23

  61  GACCCTGAATTAGTAGTACAGGAAGTACAAAGAAGCTTGAACGTGTCGCGGCGGCGACTG
      D  P  E  L  V  V  Q  E  V  Q  R  S  L  N  V  S  R  R  R  L     40
      D  P  E  L  V  V  Q  D  V  H  R  S  I  NASL T  R  R  N  L     45
                                                           *
 121  GGCTACTTGTCATGCGGCACCGGCAATCCGATCGACGACTGCTGGCGGTGCGACCCTGAC
      G  Y  L  S  C  G  T  G  N  P  I  D  D  C  W  R  C  D  P  D     60
      G  Y  L  S  C  G  S  G  N  P  I  D  R  L  L  A  M  Q  P  Q     65

 181  TGGGCTGACAACCGGCAGCGGCTCGCGGACTGCGCCATCGGGTTCGGGAAGAACGCGATT
      W  A  D  N  R  Q  R  L  A  D  C  A  I  G  F  G  K  N  A  I     80
      L  G  K  K  S  P  A  F  S  Y  C  A  I  G  F  G  K  N  A  I     85

 241  GGGGGCAGGGACGGCGAGATATACGTGGTGACCGACAGTGGCGACGACGACCCCGTCAAT
      G  G  R  D  G  E  I  Y  V  V  T  D  S  G  D  D  D  P  V  N    100
      G  G  K  N  G  R  I  Y  V  V  T  D  S  G  N  D  D  P  V  N    105

 301  CCGAAGCCGGGCACGCTCCGGTACGCCGTCATCCAGGAGGAGCCGCTGTGGATCATCTTC
      P  K  P  G  T  L  R  Y  A  V  I  Q  E  E  P  L  W  I  I  F    120
      P  K  P  G  T  L  R  H  A  V  I  Q  D  E  P  L  W  I  I  F    125

 361  AAGCGCGACATTGTCATCCAGCTGAAGGAGGAGCTCATCATGAACTCCCACAAGACCATC
      K  R  D  I  V  I  Q  L  K  E  E  L  I  M  N  S  H  K  T  I    140
      K  R  D  M  V  I  Q  L  K  Q  E  L  V  M  N  S  Y  K  T  I    145

 421  GACGGCCGGGGCGCCAGCGTCCACATCTCCGGCGGGCCGTGCATCACCATCCAGTACGTC
      D  G  R  G  A  S  V  H  I  S  G  G  P  C  I  T  I  Q  Y  V    160
      D  G  R  G  A  S  V  H  I  S  G  G  P  C  I  T  I  H  H  T    165

 481  ACCAACATCATCATCCACGGCGTCCACATCCACGACTGCAAGCAGGGCGGGAACGCGTAC
      T  N  I  I  I  H  G  V  H  I  H  D  C  K  Q  G  G  N  A  Y    180
      S  N  I  I  I  H  G  I  N  I  H  D  C  K  Q  S  G  N  G  N    185

 541  GTGCGCGACTCCCCAGGGCACTACGGGTGGCGCACGGTGTCGGACGGCGACGGGGTGTCC
      V  R  D  S  P  G  H  Y  G  W  R  T  V  S  D  G  D  G  V  S    200
      I  R  D  S  P  N  H  S  G  W  W  D  V  S  D  G  D  G  I  S    205

 601  ATCTTCGGCGGGCAGCCACCGTCTTGGGTCGACCACTGCACGCTGTTCAACTGCCACGAC
      I  F  G  G  Q  P  P  S  W  V  D  H  C  T  L  F  N  C  H  D    220
      I  F  G  G  K  -  N  I  W  V  D  H  C  S  L  S  N  C  H  D    224

 661  GGGCTCATTGACGCAATTCATGGGTCCACCGCGATCACCATTTCCAACAACTACTTGAGG
      G  L  I  D  A  I  H  G  S  T  A  I  T  I  S  N  N  Y  L  R    240
      G  L  I  D  A  I  H  G  S  T  A  I  T  I  S  N  N  Y  F  T    244

 721  CACCATGACAAGGTCATGCTGCTGGGTCACAGCGACGAGTTGACGTCCGACAAGAGCATG
      H  H  D  K  V  M  L  L  G  H  S  D  E  L  T  S  D  K  S  M    260
      H  H  D  K  V  M  L  L  G  H  S  D  S  F  T  Q  D  K  G  M    264

 781  CAGGTCACCATAGCCTTCAACCACTTCGGGGAAGACCTGGTTCAGAGGATGCCAAGGTGT
      Q  V  T  I  A  F  N  H  F  G  E  D  L  V  Q  R  M  P  R  C    280
      Q  V  T  V  A  F  N  H  F  G  E  G  L  V  Q  R  M  P  R  C    284

 841  CGGCATGGCTATTTCCACGTGGTGAACAATGACTACACCCACTGGGAGATGTACGCAATT
      R  H  G  Y  F  H  V  V  N  N  D  Y  T  H  W  E  M  Y  A  I    300
      R  H  G  Y  F  H  V  V  N  N  D  Y  T  H  W  E  M  Y  A  I    304

 901  GGTGGGAGTGCTGCTCCCACTATAAACAGCCAAGGCAACAGATTTCTTGCGCCCAATGAT
      G  G  S  A  A  P  T  I  N  S  Q  G  N  R  F  L  A  P  N  D    320
      G  G  S  A  A  P  T  I  N  S  Q  G  N  R  F  L  A  P  N  E    324

 961  CGGTTTGCAAAAGAGGTGACTAAACGCGAGGACGCACAGGAGAGCGAGTGGAAGAAGTGG
      R  F  A  K  E  V  T  K  R  E  D  A  Q  E  S  E  W  K  K  W    340
      K  Y  R  K  E  V  T  K  H  E  D  A  P  E  S  Q  W  R  S  W    344

1021  AACTGGAGGTCGGAAGGGGACCAGATGCTGAATGGAGCCTTCTTCACGCCGTCCGGGGCC
      N  W  R  S  E  G  D  Q  M  L  N  G  A  F  F  T  P  S  G  A    360
      N  W  R  S  E  G  D  L  M  L  N  G  A  Y  F  R  Q  T  G  A    364

1081  GGAGCTTCGTCGAGTCACGCGAAGGCGTCGAGCTTGGGGCCCAGGTCGTCGTCTCTCGTC
      G  A  S  S  S  H  A  K  A  S  S  L  G  P  R  S  S  S  L  V    380
      G  A  S  S  SSTY A  R  A  S  S  L  S  A  R  P  S  S  L  V    386

1141  GGGACCATCACCGTCTCTGCCGGCGTTCTCTCGTGCAAGAAGGGATCCCGCTGTTGAACC
      G  T  I  T  V  S  A  G  V  L  S  C  K  K  G  S  R  C  *       400
      G  S  I  T  T  N  A  G  P  V  N  C  K  K  G  S  R  C  *       406

1201  TCGACCACCACAAGCAGCTCAAAGCGATGCGGATATATATATCTATATATATTTATTGTG
1261  TTATCGTTGCTCATTTCTCTTCTTTCACGTACTGCATGGTTAGCCATTACGATACCAACC
1321  TCTGCCTGTTAATCTGTGAACCACTTGGGTTCAAAGTGGAAAACCCCATCTGGTTTCAGA
1381  GGCAGCCTGTGCAGATGTGTTGATACTACTTGTCTTGGGATTTGTCTAAGAATGCCTATT
1441  CAACAAAAAAAAAAAAAAAAAA  1462
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Figure 2. Nucleotide sequence and deduced amino acid sequence of the *BanI7* cDNA insert. The deduced amino acid sequence is shown below the nucleotide sequence. The stylar transmitting tissue protein from tomato encoded by the cDNA clone 9612 (Budelier et al., 1990), to which *BanI7* shows the highest homology, is included for comparison. ▲, Putative cleavage site of the N-terminal signal sequence. *, Potential Asn-linked glycosylation site present in the mature protein.

the tomato, maize, tobacco, and ragweed pollen pectate lyases, respectively. An invariant Cys residue is located at the carboxy-terminal end of *Ban*I7 and all of the other putative plant pectate lyases.

Lower but still significant homology was also found to the *pelC* and *pelE* pectate lyase genes of the bacterial plant pathogens *Erwinia carotovora* and *Erwinia chrysanthemi* (Tamaki et al., 1988; Hinton et al., 1989). A 22.9% identity and 46.8% similarity was observed for *E. chrysanthemi* pelC, whereas 23.3% identity and 44.8% similarity was found for pelE. Three main regions of homology are observed when the *Ban*I7 protein sequence is aligned with the pectate lyases from *Erwinia* spp. (Fig. 3). Two of these regions correspond to the so-called region I and region II of homology described for bacterial pectate lyases (Keen and Tamaki, 1986). Notably, these regions are the only sequences that are conserved among all *Erwinia* spp. pectate lyases, and recently have been postulated to encompass the Ca^{2+}-binding site and the putative active site of the enzyme (Henrissat et al., 1995; Yoder and Jurnak, 1995).

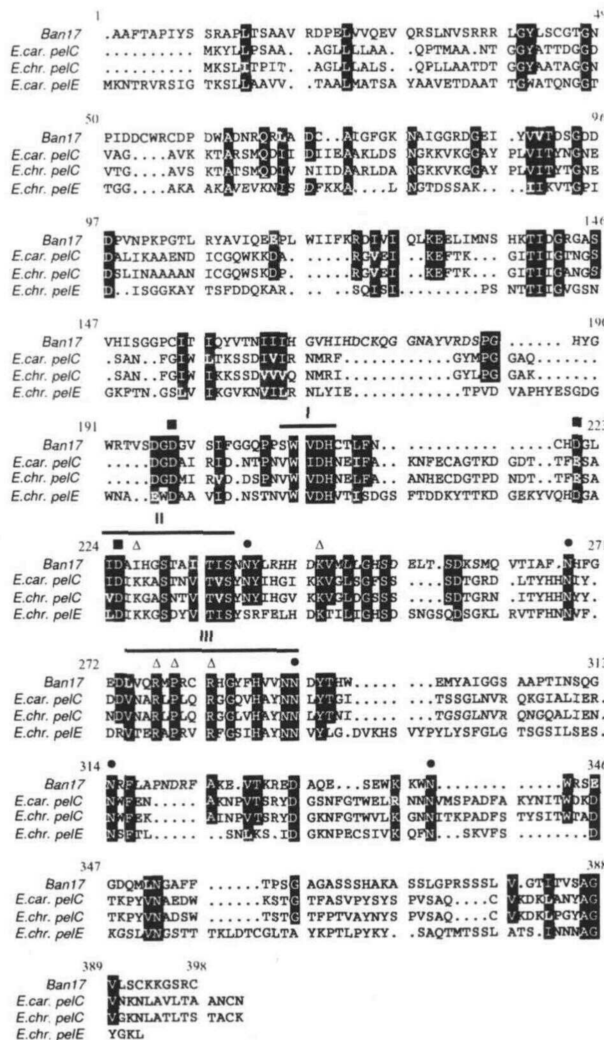

Figure 3. Amino acid sequence comparisons among the predicted protein encoded by *Ban*I7 and *Erwinia* spp. pectate lyases. Identical amino acid sequences and conservative amino acid changes are represented by black and gray boxes, respectively. Gaps were introduced to optimize alignment. The three sequence patterns (I, II, and III) conserved in all bacterial pectate lyases are shown overlined. ■, Invariant amino acids involved in Ca^{2+} coordination. △, Conserved amino acids at the putative pectate binding site. ●, Highly conserved Asn ladder. *E.car. pelC*, *E. carotovora* pelC (EMBL X16398; Hinton et al., 1989); *E.chr. pelC*, *E. chrysanthemi* pelC (EMBL M19411; Tamaki et al., 1988); *E.chr. pelE*, *E. chrysanthemi* pelE (EMBL M14509; Tamaki et al., 1988).

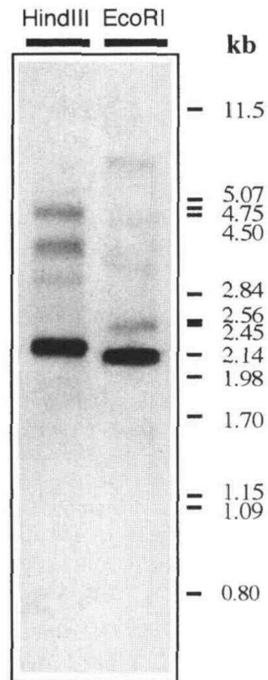

Figure 4. Southern analysis of banana genomic DNA. Genomic DNA (10 μg) was digested with *Eco*RI and *Hin*dIII and probed with the *Ban*I7 cDNA-specific probe. The sizes of marker DNA fragments run in the same gel are shown on the right.

Southern Hybridization with *Ban*I7

Banana genomic DNA was cut with the restriction enzymes *Eco*RI and *Hin*dIII and analyzed by Southern blotting. As shown in Figure 4, the *Ban*I7 probe hybridizes to one major DNA fragment and a few minor fragments. This result suggests the presence of a single gene homologous to *Ban*I7 in the banana genome. Also, the more weakly hybridizing fragments visible on Southern analysis indicate the presence of related sequences within the banana genome, which might correspond to pectate lyase homologs expressed in the pollen or the stylar transmitting tissues.

*Ban*I7 mRNA Accumulates in Green Fruit in Response to Ethylene

The onset of ripening in bananas is indicated by a sharp increase in ethylene production, followed within a few hours by a climacteric increase in respiration. Both the ethylene production peak and the respiratory peak occur as

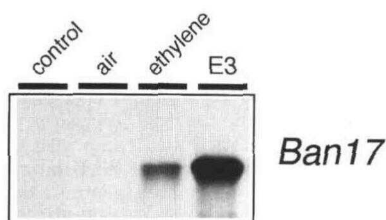

Figure 5. Effect of ethylene on *BanI7* expression in green fruit. Green banana fruits were treated with 100 parts per million ethylene in water-saturated air for 24 h (ethylene). Fruits were subjected in parallel to water-saturated air (air) as a control. RNAs were prepared from samples taken before (control) and after (air, ethylene) the treatments, and subjected to northern analysis. Fifteen micrograms of total RNA was loaded per lane and hybridized with the *BanI7* cDNA insert. RNA from climacteric fruit (E3) was included for comparison.

highly coordinated and reproducible parameters during ripening (Domínguez-Puigjaner et al., 1992), and were used to developmentally stage the fruit selected for RNA isolation (see "Materials and Methods"). This allowed us to evaluate the accumulation of *BanI7* mRNA in relation to ethylene production. *BanI7* mRNA is hardly detectable in early climacteric (E2) fruit, when ethylene synthesis has just started (see Fig. 1). Levels of *BanI7* mRNA, however, become maximal at the climacteric stage of ripening, when ethylene synthesis begins to decrease, respiration peaks, and the fruit starts to turn yellow. Therefore, the timing of *BanI7* mRNA accumulation follows the initiation of ethylene synthesis, which suggests that expression of this gene is regulated by ethylene.

To further assess the potential regulation by ethylene, green banana fruits were treated with 100 $\mu L\ L^{-1}$ ethylene for 24 h, and RNA was prepared from control and treated fruits and analyzed for *BanI7* gene expression. Figure 5 shows that *BanI7* mRNA accumulates in green fruits treated with ethylene but not in air-treated control fruits, which indicates that the accumulation of *BanI7* mRNA is regulated by ethylene.

DISCUSSION

Pectate lyase, like PG, catalyzes the cleavage of α(1–4) galacturonan linkages, but does so by a β-elimination rather than by a hydrolytic reaction mechanism. Pectate lyases are secreted by several plant pathogenic bacteria (Collmer and Keen, 1986; Kotoujansky, 1987) and, recently, multiple pectate lyase-homologs have been reported to be expressed in the stylar transmitting tissue from tomato (Budelier et al., 1990) and in the pollen grains of several plants (Wing et al., 1989; Rafnar et al., 1991; Rogers et al., 1992; Turcich et al., 1993). Here we describe the cloning and primary structure of *BanI7*, a banana ripening-specific transcript encoding a putative pectate lyase. This is the first report, to our knowledge, of a pectate lyase-related transcript in fruit.

The protein *BanI7* shares significant amino acid sequence identity (55 and 75% identity, respectively) to pectate lyases from pollen and stylar transmitting tissue. Homology to bacterial pectate lyases is substantially lower (23.3% identity to *E. chrysanthemi* pelE), but three main regions of homology are detected when the *BanI7* protein is aligned with the pectate lyases from *Erwinia* spp. (Fig. 3). Two of these regions correspond to the regions I and II described for bacterial pectate lyases (Keen and Tamaki, 1986), which were reported to be characteristic for pectate lyase activity.

The three-dimensional structures of the pectate lyases pelC and pelE of *E. chrysanthemi* have been recently reported (Lietzke et al., 1994; Yoder and Jurnak, 1995). These proteins fold in an unusual structural motif called a parallel β-helix, and detailed structural information is available on them. Ca^{+2} is essential for their in vitro pectate lyase activity, though its exact role in catalysis is not yet determined. Three Asp residues participate in Ca^{+2} coordination in bacterial pectate lyases, which are found conserved in the *BanI7* protein (Asp-197, Asp-220, and Asp-224; see Fig. 3). Surrounding the Ca^{2+} site, two invariant Arg residues (Arg-276 and Arg-281) and a Pro residue (Pro-278), thought to make up the pectate-binding site, are also conserved in *BanI7*. Homology to bacterial pectate lyases also extends to the Asn ladder, which is involved in the stabilization of the parallel β-strands and to the highly conserved vWiDH cluster (for review, see Henrissat et al. [1995]). These structural motifs are shared by pectate lyases from pollen (Wing et al., 1989; Rogers et al., 1992) and style (Budelier et al., 1990.), which suggests that all of these proteins may fold in a parallel β-helix structure and have a Ca^{2+}-dependent pectinolytic activity similar to that of bacterial pectate lyases. In fact, a polygalacturonic acid-degrading activity was recently demonstrated for the cedar pollen *Cryj I* protein (Taniguchi et al., 1995).

In banana, as in other climacteric fruits, the onset of ripening is marked by a large increase in ethylene production. This increase in ethylene appears to play a pivotal role in ripening by triggering the expression of several ripening-specific genes involved in many of the subsequent changes associated with the ripening process (Yang, 1985). A number of ripening-related mRNAs have been shown to accumulate in response to ethylene (Lincoln et al., 1987; Maunders et al., 1987). Similarly, we have shown that treatment with this hormone is able to induce the expression of *BanI7* in green banana fruits. Thus, expression of *BanI7* appears to be coordinately regulated, together with other ripening-specific genes, by the climacteric increase of ethylene at the onset of ripening.

BanI7 mRNA, on the other hand, reaches its maximum levels of expression at the respiratory climacteric peak, when changes in texture begin to take place. This pattern of mRNA accumulation is similar to that observed for PG (DellaPenna et al., 1986) and suggests a role for the *BanI7* protein in cell-wall degradation during fruit softening. The evidence for a signal peptide and a potential glycosylation site in the predicted *BanI7* protein indicates that it may enter the secretory pathway and become a part of the extracellular matrix. Experiments to assess the physiological function of *BanI7* in transgenic plants with altered levels of expression of the transcript are under way.

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