RESEARCH PAPER

Pectin esterase gene family in strawberry fruit: study of FaPE1, a ripening-specific isoform*

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Abstract

Pectin esterases (PE, EC 3.1.1.11) catalyse the demethylation of pectin. As a result of its activity, structural interactions among cell wall components during cell wall turnover and loosening are affected. In plants, PEs are typically encoded by a gene family. This family has been studied in strawberry (Fragaria x ananassa Duch.) in order to investigate the role of distinct PE genes during fruit ripening and senescence. By a combination of a PCR-based library screening and RT-PCR four different strawberry PE cDNAs, termed FaPE1 to FaPE4, have been isolated. Differential expression of each FaPE gene in various organs and during fruit development was revealed by northern blot. FaPE1 is specifically expressed in fruit, showing an increasing expression during the ripening process up to a maximum in the turning stage. Concerning hormone regulation, auxin treatment increased FaPE1 mRNA levels in green fruit, whereas exogenous ethylene decreased FaPE1 mRNA levels in ripe and senescing fruits. It is proposed that this repression of FaPE1 expression could be involved in textural changes occurring during fruit senescence.

Key words: Auxin, cell wall, ethylene, fruit ripening, pectin esterase, strawberry.

Introduction

Fruit ripening in strawberry (Fragaria spp.) is a genetically programmed process of development that, in the late stage, overlaps with senescence (Perkins-Veazie, 1995). Characteristics and composition of the ripe fruit are the result of biochemical and physiological changes. These changes include a pronounced decrease in fruit firmness that is associated with increased susceptibility to physical damage and disease. Fruit softening is mainly the result of the action of hydrolytic enzymes that modify the carbohydrate components of the cell wall. As in all non-climacteric fruits, ethylene is considered to have little or no effect on the development of the strawberry receptacle (pseudo-fruit; Perkins-Veazie, 1995). However, a role for this hormone during strawberry fruit senescence could not be excluded as it has been shown that treatment of ripe fruits with ethylene enhanced fruit softening (El-Kazzaz et al., 1983). As there is little indication that ethylene is directly involved, auxin has been proposed to be the primary hormone controlling strawberry fruit ripening. Auxin is synthesized in the achenes (true fruits embedded in the receptacle) and positively effects the initial growth of the receptacle (Given et al., 1988). A gradual decline in the supply of auxin from achenes in the latter stages of growth has been proposed to be the basis of ripening (Perkins-Veazie, 1995). It has also been reported that auxin regulates the transcription of many ripening-related strawberry genes (Manning, 1994; Medina-Escobar et al., 1997a; Aharoni et al., 2002).

Pectin is the most abundant class of macromolecule within the cell wall matrix. It is also abundant in the middle lamellae between primary cell walls, where it functions in regulating intercellular adhesion, being the major adhesive material between cells. Fruit cell walls are usually highly enriched in pectins, often more than 50% of the wall.

* The sequences reported in this paper have been deposited in the GenBank database under the accession numbers AY324809 (FaPE1), AY357182 (FaPE2), AY357183 (FaPE3), and AY357184 (FaPE4).

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Abbreviations: PE, pectin esterase; HGA, homogalacturonic acid.
During fruit softening, pectins typically undergo solubilization and depolymerization that are thought to contribute to wall loosening and disintegration (Fischer and Bennett, 1991). However, continued synthesis of cell wall polyuronides has also been suggested (Perkins-Veazie, 1995). Several pectin-modifying genes have been cloned in strawberry fruit such as pectate lyase (Medina-Escobar et al., 1997b), endopolygalacturonase (Redondo-Navedo et al., 2001) or β-galactosidases (Trainotti et al., 2001). Pectin esterase (PE) activity has also been reported in ripening strawberry fruits (Neal, 1965; Barnes and Patchett, 1976). In fruit and vegetative tissues PE occurs in multiple isoforms, and individual isoymes can be distinguished by their physical and biochemical properties (Gaffie et al., 1994; Bordenave, 1996). Molecular studies have further demonstrated that plant PEs exist in multigene families whose members have different patterns of expression. Therefore, it is speculated that heterogeneity of PE isoymes reflects divergence in functional specialization.

The cloning of four PE genes in strawberry, FaPE1, FaPE2, FaPE3, and FaPE4, is reported here. Expression of the corresponding cDNAs was examined in vegetative tissues and throughout fruit development. It was shown that the expression of FaPE1 was induced by auxin at the onset of fruit ripening and down-regulated by ethylene during fruit senescence. It is proposed that the ethylene-mediated decrease of FaPE1 expression could be an important factor determining strawberry fruit post-harvest decay.

Materials and methods

Plant materials

Strawberry plants (*Fragaria* × *ananassa* Duch.) were grown under field conditions in Huelva, in the south-west of Spain. The cultivar used was a commercial variety registered by the University of California (Davis) as Chandler. Plants of *Fragaria vesca* were provided by the *Fragaria* germplasm collection from the Churriana Center (Málaga, Spain). *Fragaria* × *ananassa* fruits were selected at seven developmental stages that had previously been established (Agius et al., 2003): F1, young flower receptacle; F2, mature flower receptacle; F3, green fruit (green receptacle and green achenes); F4, white receptacle and green achenes; F5, white receptacle with some rosy spots and brown achenes; F6, turning receptacle; and F7, red receptacle. Other tissues were stolons, roots, and expanding leaves. All analyses were performed using tissue that was harvested, immediately frozen in liquid nitrogen, and stored at −80 °C.

To analyse FaPE1 expression in senescent fruits, red ripe fruits were tagged on the vine, sprayed with an antifungal solution consisting of 0.05% carbendazim, and maintained on the vine until they showed evident symptoms of senescence. Alternatively, fruits were harvested at the ripe stage and left to overripen for 2 d at 21 °C on the laboratory bench.

Hormone treatments

In order to eliminate the endogenous auxin source, achenes were removed from F3 berries that were still attached to their parent plant. Deachenment was conducted by removing the achenes from longitudinal halves using sharp tweezers, maintaining the other half-fruit containing achenes as the control. For hormone treatment, NAA (1-naphthalametic acid) was applied to longitudinal halves of similarly staged, but otherwise intact, fruits (possessing achenes) at a concentration of 1 mM in a lanolin paste containing 1% (w/v) DMSO. Control fruits were treated solely with the lanolin–DMSO paste without NAA. Three and 5 d after deachenation and 3 d after the auxin/lanolin treatment, the two halves were separated from each other and frozen separately. A minimum of five fruits were used in each experiment.

For the ethylene treatment five F7 detached fruits were placed in 1.0 l airtight containers and subjected to a constant flow of air containing 50 μl l−1 of ethylene. Another set of F7 detached fruits were treated with 600 μl l−1 of the ethylene-perception inhibitor 1-methyl cyclopropene (1-MCP). Control (untreated detached fruits) were left in air. All treatments were performed for 24 h at room temperature.

cDNA cloning of PE from strawberry fruit

An alignment of deduced amino acid sequences corresponding to the C-terminal region of four plant pectin esterases (*L. esculentum* LePME2 (U70675); *A. thaliana* AtPME2 (U25649); *V. radiata* VrPECMEST (U94443), and *P. persica* PpPEESTR (X95991)) was used to identify two conserved domains that allowed the degenerate PCR primers to be designed. The 5’ primer FP2 [AAAGCA(C/T)GAACAGTGGCCAT/TCT(A/C/T)] corresponded to the sequence KHQAVAL and the 3’ primer RP2 [CCACTCTG(C/G)(CCA(C/T)CCTTC(A/T)GG)] to amino acids PEGWXEW.

PE-like cDNAs were cloned from a red-ripe strawberry fruit (*F. × ananassa* cv. Chandler) cDNA library prepared in the Lambda ZAP Express vector (Stratagene). Different combinations of the degenerate primers FP2 and RP2, and T3 and T7 universal primers were employed. Resulting PCR fragments were gel-purified and cloned in the pGEM-T Easy vector (Promega). The primer combination FP2 and RP2 led to *FaPE1* amplification and the reaction performed with RP2 and T7 yielded the *FaPE2* cDNA fragment.

In parallel, reverse transcriptase-PCR was performed to amplify PE transcripts expressed in immature strawberry fruit. First-strand cDNA was synthesized from 1 μg of total RNA extracted from green fruit using Expand reverse transcriptase (Roche) following the manufacturer’s instructions. The primer employed for the reverse transcription was the oligonucleotide RT1 [5’-GGCAATTCTCGAGTCGACAC(T)18-3’], which includes a poly-T sequence preceded by a 20 nucleotide sequence recognized by the primer RT2 employed in the PCR amplification. An aliquot (2 μl) of the first strand reaction was used for the subsequent PCR amplification using the primer combination FP2 (see above) and RT2 [5’-GGCAATTCTCGAGTCGACAC(C/T)3’]. The resulting PCR products were separated by gel electrophoresis, gel-purified and cloned into pGEM-T Easy vector (Promega).

Cloning of full-length FaPE1 cDNA

The full-length cDNA *FaPE1* was obtained by screening the red-ripe fruit cDNA library with the PCR fragment corresponding to the partial cDNA of *FaPE1*. The probe was radiolabelled by random priming using [α-32P] dCTP and the Klenow fragment of DNA polymerase and the screening performed using standard procedures (Sambrook et al., 1989). Seven out of 48 positive phagemids were excised in vivo by means of a helper phage and the longest one was chosen for further studies.

Isolation of FaPE1 genomic clones and promoter cloning

Genomic clones were isolated from a strawberry (*F. × ananassa* cv. Chandler) genomic library constructed in the Lambda FIX II vector
Results

Isolation of strawberry PE cDNA clones

In order to study the role of PEs during strawberry fruit development, PE cDNAs were cloned from green and ripe fruits. Degenerate primers were deduced from highly conserved PE domains and employed in RT-PCR from green fruit and in several PCR reactions using a red ripe cDNA library as template. Sequencing of the amplified fragments yielded four different cDNAs which, on the basis of comparisons with other sequences from databanks, code for different PEs named FaPE1, FaPE2, FaPE3, and FaPE4 (Fig. 1A). FaPE1 and FaPE2 were isolated from the PCR-based screen of a ripe strawberry cDNA library whereas FaPE3 and FaPE4 were obtained by RT-PCR from green stage fruit. The four cloned sequences shared a high degree of sequence similarity with PE of other plant species and possess the putative PE catalytic domain defined by Albani et al. (1991). This esterase-like domain includes the catalytic triad Asp–Asp–Arg as well as several aromatic residues that are thought to be important in the interaction with pectins (Johansson et al., 2002; Fig. 1A).

Identity and similarity percentages among the encoded amino acid sequences are shown in Fig. 1B, where it is shown that FaPE1 is the most divergent among all FaPE proteins (less than a 60% of identity) and that this isoform is highly similar to a fruit-specific PE from P. persica (PPPECSTR).

Southern analysis was conducted to determine the copy number of FaPE genes in the genome of the octoploid strawberry variety F. ×ananassa cv. Chandler. The complex pattern of bands obtained did not provide an accurate estimation of the copy number (Fig. 2) as found for other F. ×ananassa cv. Chandler genes (Trainotti et al., 1999). However, Southern analyses of F. vesca, a diploid wild relative species, indicated that each FaPE is encoded by a single gene per diploid genome (Fig. 2). This result may be suggesting that each FaPE gene from F. ×ananassa belongs to a divergent multigene family whose members are distinguishable by restriction fragment polymorphism. Interestingly, hybridization with FaPE3 and FaPE4 yielded the same band pattern with all the restriction enzymes employed, with the exception of EcoRV, since FaPE3 contains an EcoRV recognition site.

Expression pattern of the FaPE genes

The spatial and temporal expression of the four strawberry PE-related genes has been analysed by northern blot carried out under very stringent conditions (Fig. 3). FaPE1 mRNA was only detected in fruit tissue, with higher levels during the final stages of ripening. The expression of FaPE1 follows a dramatic increase from F3 to F4, coinciding with the beginning of the ripening process. During ripening FaPE1 mRNA expression shows two peaks at F4 and F6. This double peak of FaPE1 expression was routinely reproduced in a replicated northern blot experiment which included a different set of fruits. After F6, FaPE1 mRNA levels decreased although it was still detectable in red ripe fruits (F7). Like FaPE1, FaPE2 cDNA was also isolated from a cDNA library of ripe fruits. However, FaPE2 mRNA was not detected during fruit development by northern blot, its expression only being detected in leaf tissue (Fig. 3). FaPE3 and FaPE4 transcripts were detected in all the tissues analysed (Fig. 3). During fruit development FaPE3 and FaPE4 showed a similar up-down expression profile which has also been observed in other genes expressed during strawberry fruit development (Manning, 1998). However, FaPE3 seems to be more highly expressed than FaPE4 (Fig. 3). In vegetative tissues FaPE3 signal was notably more intense in leaves whereas FaPE4 expression predominated in stolons.
Isolation of FaPE1 full length cDNA and sequence analysis

A full length FaPE1 cDNA (GenBank accession number AY324809) was isolated by screening of a cDNA library with the partial FaPE1 cDNA as probe. FaPE1 cDNA is 1848 bp long with a major open reading frame extending from nucleotide 55–1599. At position −48 relative to the predicted initial ATG codon, a stop codon was found, indicating that the isolated fragment contained an entire open reading frame. The 1545 bp ORF of FaPE1 encodes a protein that shares high homology with plant PE and it is most similar (83% identical) to a PE expressed in peach fruit (PPPECESTR X95991). The amino acid sequence deduced of FaPE1 contains eight putative Asn–X–Ser/Thr glycosylation sites usually found in proteins to be secreted. The Kyte and Doolittle (1982) hydrophobicity plot (not shown) revealed the presence of a short hydrophobic domain around 25 amino acids long at the N-terminus, corresponding to the putative signal peptide and a predicted signal peptide cleavage site at Cys27 (Nielsen et al., 1997). Its removal would result in a 53.4 kDa polypeptide. Previous reports on other plant PEs have proposed an additional post-translational proteolytic cleavage (Hall et al., 1994; Bordenave et al., 1996). In the case of FaPE1 it would be expected in the vicinity of Val196,
releasing a mature protein of 35.8 kDa with a theoretical isoelectric point of 8.52. This putative mature protein which includes the esterase-like domain corresponds to the carboxy-terminal region of the precursor and its molecular mass is similar to that of the 33.5 kDa PE recently purified from ripe strawberry fruit (Nguyen et al., 2002a).

**Effect of auxin on FaPE1 expression**

The role of auxin during fruit development was analysed in F3-stage fruits. At this stage, the endogenous peak of auxin occurs in the berry (Archbold and Dennis, 1984) and it also precedes an increase in the expression of FaPE1. Because it is well established that achenes are a rich source of auxins, in order to test whether FaPE1 mRNA expression is affected by the removal of endogenous auxin, northern blot was conducted in deachened green fruits. As shown in Fig. 4A, FaPE1 mRNA expression was undetectable in fruits 3 d after the elimination of the achenes. By contrast, FaPE1 mRNA levels were higher in tissue with achenes 3 and 5 d after the treatment, suggesting that auxin is

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**Fig. 2.** Genomic Southern blot analysis of DNA from the strawberry species *F. × ananassa* and *F. vesca*. Genomic DNA (10 μg per lane) was digested with the indicated restriction enzymes and hybridized with 32P-labelled fragments of FaPE1–FaPE4 probes.
required for FaPE1 expression during fruit ripening. To
examine the auxin effect further, green fruit halves were
covered with a lanolin paste with or without NAA.
Northern blot analysis showed that NAA induces an
increase in FaPE1 expression 3 d after the auxin applica-
tion compared with the untreated control (Fig. 4B). Fruits
treated with lanolin did not show changes in FaPE1
mRNA abundance (Fig. 4B). As a control for the auxin
treatment, the same blots were stripped and subsequently
hybridized with a probe for FaPE3 and no changes in gene
expression were observed (data not shown).

Ethylene regulation of FaPE1 expression
To gain insight into the regulation of FaPE1 gene
expression the promoter region of FaPE1 was isolated
from a F. ×ananassa genomic library constructed in λ FIX
II. Library screening was performed using the partial
FaPE1 cDNA as probe. An amplified fragment of 1.6 kb
was cloned and sequenced; its identity was confirmed by
identical overlapping with the 5′ UTR sequence of FaPE1
cDNA. The 1.6 kb fragment included a putative TATA box
located at nucleotide −145 relative to the ATG initiation
codon. Sequence analysis of the FaPE1 promoter region
using PlantCare software (Rombauts et al., 1999) led to the
identification of putative cis-acting elements that could be
involved in FaPE1 transcriptional regulation. Three
ethylene responsive elements (EREs) were located at
positions −386, −812, and −1065 (Fig. 5A). Noticeably, no
auxin-responsive elements were found in the 1.6 kb region
analysed. Each ERE consists of an 8 bp motif with the
sequence A(A/T)TTCAAA and they have previously been
identified in the promoter region of ethylene-stimulated
genes (Montgomery et al., 1993; Itzhaki et al., 1994).

Fig. 3. Northern hybridization analysis of pectin esterase gene expression during strawberry fruit development (F1–F7), and in stolons (S), leaves (L), and roots (R). 15 μg of total RNA were used in these analyses.

Fig. 4. Effect of auxins on FaPE1 expression. Northern blot analysis
of total RNA (15 μg) extracted from green fruits under different auxin
conditions. (A) FaPE1 expression in the presence (+Ach) or absence
(−Ach) of achenes 3 d and 5 d after deachenation. (B) FaPE1
expression in untreated (−NAA) and 3 d after the application of 1 mM
NAA in a lanolin paste (+NAA). As a control for the auxin treatment,
another set of fruits were treated with the lanolin paste only (+Lan) or
left untreated (−Lan).

To test the biological significance of the putative EREs
in the promoter of FaPE1, the effect of the hormone
ethylene in FaPE1 expression was analysed. Treatment
was performed in F7 strawberry fruits, coinciding with an
endogenous peak of ethylene production (Iannetta et al.,
2000). When treated for 24 h with 50 μl l⁻¹ ethylene, fruits
showed a decrease in FaPE1 mRNA accumulation compared with the control air-treated fruits (Fig. 5B). F7 fruits were also treated with the inhibitor of ethylene action 1-methyl cyclopropene (1-MCP). After 24 h of treatment, FaPE1 mRNA levels were higher in 1-MCP treated fruits (Fig. 5C), thus strongly supporting the fact of a negative regulation of FaPE1 expression exerted by the hormone ethylene.

FaPE1 expression in senescing strawberry fruits

FaPE1 expression was also analysed in overripe fruits. Firstly, several fruits were harvested at the ripe stage (F7) and left to overripen on the shelf for 24 or 48 h at 21 °C. A second set of fruits were maintained on the vine and harvested after 48 h and several days later when fruits showed clear symptoms of senescence i.e., a semi-melted texture. During senescence, fruits that were maintained on the plant showed a progressive reduction in FaPE1 transcripts, being undetectable at the overripe stage (Fig. 6). Similarly, in detached fruits maintained at room temperature FaPE1 mRNA levels also decreased. However, the kinetics of the process was faster and FaPE1 transcripts were hardly detected after 24 h (Fig. 6).

Discussion

Changes in the pectin matrix are regarded as an important factor that affects cell wall structure during ripening and senescence. In this context, PE activity is a key control point for both the assembly and disassembly of pectin networks. Homogalacturonic acid (HGA) is an abundant and widespread component of the polysaccharide complex, commonly termed ‘pectin’, that appears to be synthesized in the Golgi apparatus in a highly methyl-esterified state (Doong et al., 1995). Subsequent to its incorporation into the extracellular matrix, HGA often become enzymatically de-esterified by the action of the enzyme PE. De-esterification of HGA increases the negative charge density in the cell wall microenvironment. Therefore HGA can be cross-linked by divalent cations, such as calcium, resulting in supramolecular assemblies and gels. The formation of these calcium-mediated pectin gels significantly affects the mechanical properties of the cell wall and adds rigidity to the wall (Jarvis, 1984; Willats et al., 2001). However, the degree and pattern of methyl esterification is also important in regulating the cleavage of HGA by pectinolytic enzymes, such as polygalacturonase and pectate lyase, and may influence the activities of other wall-associated enzymes by altering the pH of the local environment (Pressey and Avants, 1982; Almeida and Huber, 1999).

In this paper, the existence of four strawberry PE-related genes is reported, three of them expressed during fruit development at a level that could be detected by northern blot hybridization. The occurrence of multiple isoforms of PE in strawberry fruit was expected as several PE isoforms have previously been described in different fruits such as tomato, peach, and banana (Hall et al., 1994; Glover and Brady, 1994; Nguyen et al., 2002b), and also in a strawberry fruit EST collection (Aharoni and O’Connell, 2002). It is possible that the different genes may encode proteins with slightly different functions in the cell wall or temporally associated with a particular cell type, tissue or organ through plant development. Each FaPE gene showed a unique expression profile and it was possible to classify them into functionally distinct groups as determined for other PE gene families (Micheli et al., 1998; Gaffe et al., 1994). The first FaPE group comprises genes showing an organ-specific expression, at least at a level detectable by northern blot; this includes FaPE1 in ripening fruit and FaPE2 in leaves. Ripening stages where FaPE1 expression were detected coincided with the PE activity profile described by Barnes and Patchett.
(1976), starting from small green berries up to the overripe berries. Maximum activity was found around ripening time and, like FaPE1 mRNA, the activity decreased at the overripe (senescent) stage, suggesting that FaPE1 activity highly contributes to the overall PE activity during fruit development. FaPE3 and FaPE4 belong to the second group, which includes those FaPE genes that are ubiquitously expressed throughout the plant. Thus, FaPE3 is most identical (82%) to PMEU1 (AY046596), another ubiquitous PE from tomato (Gaffe et al., 1997). The expression pattern of this last group of PE genes supports the existence of ‘house-keeping’ PEs involved in maintaining cell wall integrity throughout plant development (Micheli et al., 1998). In strawberry, up to four PE-like ESTs have been reported whose expression was followed by DNA microarrays (Aharoni and O’Connell, 2002). They were receptacle-specific and transcript level varied along fruit ripening stages. Sequence information is available only on two of them (CB934832, CB934833) and did not show similarity to any of the strawberry cDNAs here reported.

Southern blot analysis of genomic DNA from F.×ananassa, an octoploid species, and that from the diploid species F. vesca was conducted. In the genome of the diploid species each FaPE represents a single-copy gene. It can be observed that all the bands in F. vesca seem to be a subset of the bands detected in F.×ananassa. This fact agrees well with the genetic origin of F.×ananassa. It is known that a diploid set of the eight genomes that comprise the species F.×ananassa is homologous to the modern diploid species which includes F. vesca (Senanayake and Bringhurst, 1967). Thus, the elevated allele polymorphism observed in F.×ananassa may reflect divergence among the F.×ananassa progenitors. The parallel band patterns using the FaPE3 and FaPE4 probes suggest that these genes may be in a tandem array. In tomato, PE genes have also been reported to be present in the genome as a tandem repeat (Hall et al., 1994).

The control of ripening in non-climacteric species, such as strawberry, is poorly understood. In strawberry it has been shown that auxin delays ripening by altering the expression of many ripening-associated genes (Given et al., 1988). Most of the strawberry ripening-related genes are negatively regulated by auxin, although a few auxin up-regulated genes have also been described (Manning 1998; Aharoni et al., 2002). This last report (Aharoni et al., 2002) included a pectin esterase-like EST (H163, CB934833) whose short sequence did not show similarity to FaPE1. It is known that free and conjugated indole-3-acetic acid (IAA) reaches a peak at the green fruit stage of fruit development and subsequently declines (Archbold and Dennis, 1984). Removal of the endogenous auxin source in halved green fruit resulted in a reduced FaPE1 transcript accumulation, whilst exogenous auxin application caused an enhancement of FaPE1 expression.

Strawberry is considered a non-climacteric fruit, however, a small increase in ethylene production has been reported at late ripening stages (Perkins-Veazie et al., 1996; Iannetta et al., 2000). An in vivo role for ethylene is supported by the finding that exogenous application of ethylene accelerates strawberry fruit senescence and causes a 50% decrease in fruit firmness (El-Kazzaz et al., 1983). Recently, Jiang et al. (2001) also demonstrated that 1-MCP treatment of ripe strawberry fruits tended to maintain fruit firmness and lowered increases in anthocyanin and phenolic contents. The observed changes in fruit firmness could be influenced by changes in FaPE1 activity. The promoter region of the FaPE1 gene has been shown to contain three putative EREs. This element has previously been found in ethylene-responsive promoters such the carnation GST and the tomato E4 gene promoters (Montgomery et al., 1993; Itzhaki et al., 1994). The responsiveness of the FaPE1 promoter region to ethylene was proven, as FaPE1 mRNA levels decrease after ethylene treatment in ripe fruits. Inhibition of ethylene perception with 1-MCP resulted in an increase of FaPE1 transcripts, also supporting a role for ethylene in regulating FaPE1 expression. Although auxin is known to regulate the expression of many ripening-related strawberry genes, as far as the authors are aware this is the first time that an ethylene-responsive gene is characterized in a non-climacteric fruit such as strawberry. Previously, some other ethylene-responsive genes have also been reported in non-climacteric fruits (Alonso and Granell, 1995; Cazzonelli et al., 1998). However, the effect of this hormone has not been described for any strawberry gene. In particular, both an expansin (FaExp2) and a cellulase (FaCell) appeared to be ethylene-insensitive (Civello et al., 1999).

It is possible that the repression of FaPE1 mRNA expression at the late stages of fruit ripening and, in particular during senescence, could be an important factor determining the post-harvest life of the strawberry fruit. In strawberry (Barnes and Patchett, 1976), as in many other fruits such as peach (Glover and Brady, 1994), banana (Kanellis et al., 1989), and melon (Filis-Lyacon and Buret, 1991), a decrease in total PE activity has been observed at the end of the ripening process, overlapping with senescence. In tomato fruit, the role of pectin esterase in fruit softening has been studied by constitutive expression of a fruit-specific PE antisense gene (Tieman et al., 1992). Normal ripening occurred in the transgenic plants with reduced PE activity, but there was a significant decrease in tissue integrity during fruit senescence. Cell walls from these fruits with a lower degree of methyl esterification showed an altered cation-binding capacity as a consequence of the lower frequency of anionic charges on fruit pectins (Tieman and Handa, 1994). The role of the tomato fruit-specific PE isoform that affects tissue integrity during senescence (Brummell and Harpster, 2001) could be
common to FaPE1. During strawberry fruit senescence, the inhibition of FaPE1 following the endogenous ethylene burst would increase the degree of methyl esterification of pectic polysaccharides and thus reduce pectin stabilization mediated by calcium cross-links. The inability to form calcium cross-bridges would lead to cell separation and have a negative effect on fruit integrity. In agreement with this proposal, the calcium content of many fruits has been related to the quality of fruit in storage. In the case of strawberries it has been proven that dips in calcium delay fruit post-harvest decay and maintain its firmness (García et al., 1996).

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References


 Kanellis AK, Solomos T, Mattoo AK. 1989. Changes in sugars, enzymic activities, and acid phosphatase isoenzyme profiles of bananas ripened in air or stored at 2.5% O2 with and without ethylene. *Plant Physiology* 90, 251–258.

Kyte J, Doolittle RF. 1982. A simple method for displaying the


