Ethylene-regulation of fruit softening and softening-related genes in peach

Hiroko Hayama¹,*, Takehiko Shimada², Hiroshi Fujii², Akiko Ito¹ and Yoshiki Kashimura¹

¹ National Institute of Fruit Tree Science (NIFTS), 2-1 Fujimoto, Tsukuba, Ibaraki 305-8605, Japan
² Okitsu Citrus Research Station, NIFTS, 485-6 Okitsunakamachi, Shimizu, Shizuoka, Shizuoka 424-0292, Japan

Received 4 July 2006; Accepted 30 August 2006

Abstract
To investigate the role of ethylene in peach fruit softening during ripening, stony hard peach fruit, in which ethylene production is suppressed during ripening, were treated with various concentrations of ethylene. There was no noticeable decrease in flesh firmness without ethylene treatment, while applied ethylene, in the range 0.1–100 μl l⁻¹, resulted in fruit softening. Furthermore, the fruit softened more rapidly when the applied ethylene concentration was higher. When ethylene treatment was interrupted, the degree of softening was greatly reduced. These results indicated that continuous ethylene treatment was required for the initiation and progression of fruit softening and that ethylene concentration is also an important factor in regulating the rate of softening. Eight genes, which putatively encode cell wall metabolism-related proteins, were investigated for mRNA accumulation patterns in the two different softening phenotypes of melting and stony hard peaches. All of the mRNAs investigated accumulated in fruit of the melting-flesh ‘Akatsuki’ during ripening. By contrast, in the stony hard-flesh ‘Manami’, the mRNAs for a putative endopolygalacturonase (PpPG), an α-L-arabinofuranosidase/β-xylosidase (PpARF/XYL), and an expansin (PpExp3) showed either much lower levels or did not accumulate, and were identified as softening-related genes. Interruption of ethylene treatment indicated that these genes were regulated at the transcriptional level, and quickly responded to the presence or absence of ethylene before the softening response occurred, suggesting that ethylene directly regulates the transcription of these softening-related genes. These results suggested that cell wall metabolism, causing a rapid loss of firmness in peach fruit, may be controlled by ethylene at the transcriptional level.

Key words: Cell wall, ethylene, fruit ripening, peach, Prunus persica, softening, transcription.

Introduction
Fruit softening during ripening is a complex process that occurs as a result of numerous modifications of the cell wall polysaccharide architecture. As fruit texture is unique in different fruit species, the modification and structure of cell walls are also different. Several ripening-related cell wall modifications, such as the solubilization or depolymerization of pectin or other cell wall components, are observed in some fruits, however, the extent and the timing of these modifications vary (Brummell, 2006). As most of the disassembly or the modification of cell wall polysaccharides during ripening is due to the actions of a range of cell wall-modifying enzymes, the activities of these enzymes also differ widely among species (Yoshioka et al., 1992; Orr and Brady, 1993). In peach fruit, the activities of several enzymes have been shown to increase during softening: exopolygalacturonase (exoPG, EC 3.2.1.67), endopolygalacturonase (endoPG, EC 3.2.1.15), endo-1,4-β-mannanase (EC 3.2.1.78), α-L-arabinofuransidase (ARF, EC 3.2.1.55), and β-galactosidase (EC 3.2.1.23) (Pressey and Avants, 1973; Brummell et al., 2004).

In climacteric fruits, an increase in ethylene production is observed before the initiation of ripening, and ethylene is a trigger of the ripening process (Oeller et al., 1991). However, not all of the ripening-related events are dependent on ethylene; some are controlled by other hormonal and/or developmental factors. Both the ethylene-dependent...
and ethylene-independent pathways coexist to co-ordinate the ripening process (Lelièvre et al., 1997; Alexander and Grierson, 2002). Transgenic melon fruit, in which ethylene synthesis was suppressed by an antisense 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase construct, were used to show that softening depended partially on ethylene and displayed some ethylene-independent components (Flores et al., 2001). By contrast, transgenic tomato fruit in which endogenous ethylene production was suppressed by the expression of an antisense ACC synthase, did not soften. In these fruits, exogenous propylene or ethylene reversed the antisense phenotype, indicating that softening was completely dependent on ethylene (Oeller et al., 1991).

Peach fruit are climacteric, and an increase in ethylene production is observed during fruit ripening (Miller et al., 1988; Tonutti et al., 1991, 1996). However, the relationship between ethylene and fruit softening is unclear, because peach fruit softening begins significantly earlier than the increase in ethylene production (Tonutti et al., 1996). Furthermore, 1-methylecyclopropene (1-MCP), a strong ethylene action inhibitor, is effective in slowing softening, but this inhibitory effect occurs only while the inhibitor is present, and for 12–24 h after the end of the treatment (Rasori et al., 2002). Fruit treated with 1-MCP began to soften 1–2 d after the treatment ended (Rasori et al., 2002; Liguori et al., 2004). In other climacteric fruits such as apples, pears or plums, 1-MCP delayed softening for some weeks after treatment (Fan et al., 1999: Hiwasa et al., 2003; Menniti et al., 2004). Therefore, the exact relationship between ethylene and fruit softening during ripening is not fully understood.

The stony hard peach cultivar ‘Manami’ is an ideal mutant for studying the relationship between fruit softening and ethylene. In this mutant, the ripe fruit produce little ethylene and firm flesh is maintained during ripening, but exogenous ethylene induces a rapid loss of firmness (Haji et al., 2003; Hayama et al., 2003). Recently, it was reported that the suppression of fruit softening in stony hard peach is caused by reduced expression of PpACS-1, a member of the ACC synthase family, only at the fruit ripening stage, and that the ripe fruit generate almost no ethylene autocatalytically after ethylene treatment (Tatsuki et al., 2006). Furthermore, the softenings of stony hard peach fruit, promoted by ethylene treatment, has been shown to be accompanied by increases in both endoPG and exoPG activities (Hayama et al., 2006).

In this study, eight putative cell wall-related genes that are expressed in ripe peach fruit were analysed and three genes were identified, putatively encoding an endoPG, an α-L-arabinofuranosidase (ARF, EC 3.2.1.55)/β-xylosidase (XYL, EC 3.2.1.37), and an expansin (PpExp3), whose transcripts accumulated to higher levels in fruit of the melting flesh cultivar than those of the stony hard flesh cultivar. The accumulation of mRNA from these three genes, as well as peach fruit softening, were completely ethylene-dependent, suggesting that ethylene controls peach fruit softening by the transcriptional regulation of these genes. The effects of altering ethylene concentration, or interrupting exogenous ethylene treatment, were also investigated.

Materials and methods

Plant material and ethylene treatment

Fruit of peach (Prunus persica (L.) Batsch) cvs ‘Akatsuki’ and ‘Manami’ were hand picked at the commercial harvesting time from trees planted at the NIFTS, Ibaraki and a commercial orchard in Nagano, Japan, respectively. They were packed in 40 l containers and subjected to a continuous flow of carbon dioxide-free and humidified air at a flow rate of 7.0 l min⁻¹ at 25 °C. For ethylene treatment of ‘Manami’ fruit, ethylene was added to the air flow to a final concentration of 0, 0.1, 1.0, 10, or 100 μl l⁻¹. Ethylene and carbon dioxide levels in the containers were checked regularly according to Hayama et al. (2006). For the investigation of the effects of the interruption of ethylene treatment, fruit were treated for 2 d then taken out of the containers and stored in ethylene-free air at 25 °C. Flesh tissue was diced and immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

Measurement of flesh firmness

Fruit flesh firmness was measured after removing small discs of skin from the centres of the two halves of the fruit, on either side of the suture. The force required to insert a penetrrometer (FT011, Fujihr Industry Co. Ltd, Tokyo, Japan), with a plunger of 8 mm diameter, into the flesh was determined.

RNA extraction and cDNA isolation

Total RNA was extracted from mesocarp tissue of peach fruit by a hot-borate method (Wan and Wilkins, 1994). A subtraction library was constructed using a PCR-selected™ cDNA subtraction library construction kit (Clontech, Mountain view, CA, USA). The tester was mRNA extracted from ‘Manami’ fruit after 2 d of ethylene treatment, and the driver was mRNA from ‘Manami’ flesh after a 2-d treatment with ethylene-free air. The subtractive cDNAs were inserted into the T7 vector (Novagen, Darmstadt, Germany). Another cDNA library (the ME library) was constructed from ‘Manami’ fruit after 2 d of ethylene treatment, using a ZAP-cDNA Synthesis kit (Stratagene, La Jolla, CA, USA) and cloned into the UniZAP XR vector. Phage clones were packaged using a Gigapack Gold II packaging kit (Stratagene). The cDNAs were excised as Bluescript SK+ plasmids (Stratagene) in the SOLR bacterial host strain according to the in vivo excision protocol supplied by Stratagene. In total, 107 and 1152 cDNA clones were randomly selected from the subtraction library and the ME library, respectively, and sequenced using an ABI prism sequencing kit and an ABI370 sequencer (Applied Biosystems, Foster City, CA, USA). Sequence similarity searches were performed using the Basic Local Alignment Search Tool (BLASTN, National Center for Biotechnology Information, Bethesda, MD, USA). Putative signal sequence peptides were identified using the SignalIP version 3.0 web server.

Out of the randomly sequenced cDNA clones, five that encoded putative cell wall-related enzymes: endoPG (Clone no. SB2101), endo-1,4-glucanase (EG, EC 3.2.1.4;Clone no. SB4012), ARF/XYL (Clone no. ME0463), and two pectate lyases (PL, EC 4.2.2.2;Clone nos ME1022 and ME0380) were selected for further analysis. Selected clones were sequenced and used in RNA gel blot analyses, along with three previously isolated expansin genes: PpExp1, PpExp2, and PpExp3 (Hayama et al., 2000, 2003).
**RNA gel blot analysis**

RNA gel blot analysis was as in Hayama et al. (2003) with some modifications. Total RNA (5 μg lane−1) was separated by electrophoresis in 1.2% (w/v) agarose/0.66 M formaldehyde gels and transferred to nylon membranes (Hybond N+, Amersham Biosciences, Piscataway, NJ). Membranes were prehybridized for 2 h in high SDS buffer [50% (v/v) deionized formamide, 5× SSC, 7% (w/v) SDS, 2% (w/v) blocking reagent (Roche Diagnostics, Basel, Switzerland), 0.1% (w/v) N-lauroylsarcosine, 50 mM sodium phosphate pH 7.0] and hybridization was performed overnight in the same buffer containing a DIG-labelled probe at 50 °C. Following hybridization, membranes were washed twice with 2× SSC containing 0.1% (w/v) SDS for 5 min at room temperature, and twice with 1× SSC containing 0.1% (w/v) SDS for 15 min at 68 °C. Signals were detected by chemiluminescence using CSPD™ (Roche Diagnostics) and exposed to X-ray film (Fuji Photo Film, Tokyo, Japan).

Probes for eight cell wall-related genes (Table 1) were prepared with a PCR DIG probe synthesis kit (Roche Diagnostics) according to the manufacturer’s instructions. In order to differentiate between the expression patterns of two pectate lyase genes, specific probes containing the 3′-untranslated regions of each PL gene (nt. 1253–1725 for ME1022, and nt. 1216–1959 for ME0380) were generated by PCR. For each probe, the T7 primer was used as the downstream primer, and nt. 1216–1959 for ME0380) were generated by PCR. The 3′-untranslated regions of each PL gene (nt. 1253–1725 for ME1022, and nt. 1216–1959 for ME0380) were generated by PCR. For each probe, the T7 primer was used as the downstream primer, and the gene-specific upstream primers were: 5′-GGCAAGGC-CATCTTCTCTAGTG-3′ for ME1022, and 5′-ATGTTGGAAGCATAACTTCCAG-3′ for ME0380. Specific probes for PpExp1, PpExp2, and PpExp3 were the produced as described previously (Hayama et al., 2003).

**Results**

**Sequence analysis**

In order to isolate fruit softening-related genes, two different cDNA libraries, the subtraction library and the ME library, were prepared and clones were randomly chosen for sequencing. From the subtraction library, two cDNA clones (SB2101 and SB4012), homologous to genes encoding an endoPG and an EG, respectively, were obtained from the 107 clones that were sequenced (Table 1). SB2101 was a partial cDNA of 515 bp, containing the 3′ end of the coding region, encoding 55 amino acids of the endoPG, and 350 bp of the 3′ untranslated region. It showed high sequence similarity, with an E-value of 0.0, to a peach endoPG (Accession no. X76735), which is responsible for a melting/non-melting locus (Callahan et al., 2004), and was named PpPG. SB4012 was 551 bp in length and contained a partial coding sequence, lacking both the 5′ and 3′ ends. The clone was identified as a peach EG (PpEG4, Accession no. AJ890498), and was named PpEG4.

From the ME library, a total of 1152 clones were sequenced, and several cell wall-related genes were identified. The class of cell wall-related clones that was isolated most commonly (i.e. 26 times) encoded proteins homologous to PL. Sequence analysis indicated that these clones could be classified into two distinct sequences. Clones ME1022 and ME0380 contained the longest coding regions, and were used as a representative clones for these two PL sequences (Table 1). ME1022 showed high sequence similarity with a PL from Prunus mume (Pm65, Accession no. AB218786). It was 1725 bp in length, with an open reading frame encoding 413 amino acids, including a 29 amino acid signal peptide at the 5′ end. ME0380 was 1959 bp in length and encoded a putative polypeptide of 425 amino acids. The clone appeared to contain an almost complete gene sequence, but lacked several amino acids at the 5′ end. ME0380 was most homologous to a PL isolated from Fragaria x ananassa. Since ME1022 and ME0380 both contained the coding regions of PL genes, they were named PpPL1 (Accession no. AB264095) and PpPL2 (Accession no. AB264096), respectively. For distinguishing between these two genes on RNA gel blots, specific probes were constructed using non-conserved regions of these sequences.

The clone ME0463 (1077 bp) showed high sequence similarity to a peach putative β-xylosidase (PpAzl52, Accession no. AF362990), which has been shown to be an ethylene-responsive gene (Ruperti et al., 2002). ME0463 was also very similar, with an E-value of 2e-140, to an ARF (PpARF2, Accession no. AB195230) expressed in ripe fruit of Japanese pear (Tateishi et al., 2005). As ME0463 showed high sequence similarity with both the ARF and the XYL, it was named PpARF/XYL (Accession no. AB264280).

**Table 1. List of cDNA clones used in this study**

The column ‘Putative function’ refers to the function or putative function of the matched gene sequence with the highest BLASTN score. The ‘Organism’ is the organism from which the matched sequence was derived. The ‘Score’ is the alignment score in bits, and the ‘E-value’ provides an estimate of statistical significance for the alignment.

<table>
<thead>
<tr>
<th>Clone name</th>
<th>Clone no.</th>
<th>Putative function</th>
<th>Organism</th>
<th>Accession no.</th>
<th>Score (bits)</th>
<th>E-value</th>
<th>Identity (%)</th>
<th>Overlap (bp)</th>
<th>Clone length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PpPG</td>
<td>SB2101</td>
<td>Endopolygalacturonase</td>
<td><em>Prunus persica</em></td>
<td>X76735</td>
<td>769</td>
<td>0.0</td>
<td>97</td>
<td>431</td>
<td>515</td>
</tr>
<tr>
<td>PpARF/XYL</td>
<td>ME0463</td>
<td>β-1,4-β-glucanase</td>
<td><em>Prunus persica</em></td>
<td>AF362990</td>
<td>2004</td>
<td>0.0</td>
<td>98</td>
<td>1059</td>
<td>1077</td>
</tr>
<tr>
<td>PpPL1</td>
<td>ME1022</td>
<td>Pectate lyase</td>
<td><em>Prunus mume</em></td>
<td>AB218786</td>
<td>2385</td>
<td>0.0</td>
<td>98</td>
<td>1298</td>
<td>1725</td>
</tr>
<tr>
<td>PpPL2</td>
<td>ME0380</td>
<td>Pectate lyase</td>
<td><em>Fragaria x ananassa</em></td>
<td>AF339025</td>
<td>844</td>
<td>0.0</td>
<td>91</td>
<td>642</td>
<td>1959</td>
</tr>
<tr>
<td>PpEG4</td>
<td>SB4012</td>
<td>Endo-β-1,4-glucanase</td>
<td><em>Prunus persica</em></td>
<td>AJ890498</td>
<td>1092</td>
<td>0.0</td>
<td>100</td>
<td>551</td>
<td>551</td>
</tr>
<tr>
<td>PpExp1</td>
<td>–</td>
<td>Expansin (PpExp1)</td>
<td><em>Prunus persica</em></td>
<td>AB029083</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PpExp2</td>
<td>–</td>
<td>Expansin (PpExp2)</td>
<td><em>Prunus persica</em></td>
<td>AB047518</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PpExp3</td>
<td>–</td>
<td>Expansin (PpExp3)</td>
<td><em>Prunus persica</em></td>
<td>AB047519</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
RNA gel blot analysis of cell wall-related genes in two different fruit textures during ripening

The mRNA accumulations patterns were investigated using two cultivars with different fruit flesh textures: ‘Akatsuki’, a melting type which rapidly softens during ripening, and ‘Manami’, a stony hard type which remains firm during ripening. The flesh firmness of both ‘Akatsuki’ and ‘Manami’ fruit at harvest was approximately 29 Newton (N). Thereafter, ‘Akatsuki’ fruit rapidly lost their firmness within 2 d, while ‘Manami’ fruit remained firm during the ripening period of 5 d (Fig. 1A). In the melting-flesh of ‘Akatsuki’, the transcripts of all genes investigated in this study were detected during ripening (Fig. 1B). The mRNAs for PpPL1, PpPL2, PpExp2, and PpExp3 were expressed constitutively throughout the ripening period of 5 d. Levels of PpPG and PpARF/XYL mRNA increased during ripening, while PpEG4 and PpExp1 mRNA levels decreased. The levels of mRNA for PpPG, PpARF/XYL, and PpExp3 were much less abundant in the firm flesh ‘Manami’ cultivar than in ‘Akatsuki’, although the other genes: PpPL1, PpPL2, PpEG4, PpExp1, and PpExp2, were expressed at similar levels in both fruit types.

Effects of ethylene on flesh firmness

In order to clarify the effects of ethylene on fruit softening in peach, fruit of the stony hard cultivar ‘Manami’ were treated with different concentrations of ethylene (0, 0.1, 1, 10, and 100 μl l⁻¹). There was no noticeable decrease in flesh firmness after 14 d with a continuous flow of ethylene-free air (Fig. 2). When ethylene concentrations ranging from 0.1 to 100 μl l⁻¹ were applied, higher concentrations of ethylene reduced the flesh firmness more rapidly (Fig. 2). When the ethylene treatment was interrupted after 2 d, the softening process was repressed approximately 0.5–1 d later. In fruit treated with higher concentrations of ethylene, the delay between the interruption in ethylene treatment and the repression of fruit softening was longer (i.e., approximately 1 d; Fig. 2). These results indicated that a continuous presence of ethylene was required for fruit softening to occur, and that the amount of ethylene affected the degree of softening in peach.

Transcriptional regulation of softening-related genes by exogenous ethylene in stony hard peaches

Three genes that were expressed at much lower levels in the stony hard-flesh ‘Manami’ cultivar than in the melting-flesh ‘Akatsuki’ cultivar (Fig. 1B) were induced by ethylene, and detected in ethylene-treated ‘Manami’ fruit (Fig. 3). PpPG mRNA accumulated to higher levels with higher concentrations of ethylene treatment, and quickly decreased after treatment with exogenous ethylene was suspended. PpARF/XYL transcripts were detectable after treatment with 10 μl l⁻¹ or more of ethylene, and they disappeared promptly when ethylene treatment was suspended. PpExp3 mRNA accumulation was induced by a lower level of ethylene than the levels required by the other two genes, and its response to the suspension of ethylene treatment was slower. Thus, the accumulation of all of these three mRNAs responded to the presence or absence of exogenous ethylene within 0.5–1 d, although the ethylene sensitivities of the three genes varied. These results indicated
that the expression levels of softening-related genes were tightly regulated by ethylene, and that the continuous presence of ethylene was required to maintain their mRNA accumulation.

Discussion

In climacteric fruit including peach, ethylene is considered to be a trigger of fruit-ripening events, including softening. However, it is unclear how and when ethylene regulates fruit softening in peach. Stony hard peach is an ideal mutant for studying the role of ethylene in ripening events, because the ripe fruit produce little ethylene even after ethylene treatment (Tatsuki et al., 2006), and the effects of applied ethylene treatment are not masked by the initiation of autocatalytic ethylene production. In this study, the continuous application of ethylene to stony hard peach, or the cessation of ethylene treatment after 2 d, clearly indicated that ethylene is required for both the initiation and progression of fruit softening. Furthermore, the fruit soften more rapidly when the applied ethylene concentration is higher, ranging up to 100 $\mu$L $^{-1}$, suggesting that the amount of ethylene is an important factor for determining the rate of softening in peach.

Changes in the cell wall during peach fruit softening are very complex, and a number of cell wall-related genes and proteins are involved in the different sets of cell wall modifications (Trainotti et al., 2003; Brummell et al., 2004). A total of eight genes that encode putative cell wall-related
proteins were isolated from ripe peach fruit and investigated in this study. Among them, the expression levels of PpPG, PpARF/XYL, and PpExp3 were correlated with the degree of fruit softening, and these genes were identified as softening-related genes. PpPG and PpExp3 have been shown to be involved in peach fruit softening in previous studies using non-melting peach (Callahan et al., 2004) and stony hard peach (Hayama et al., 2003), while PpARF/XYL was newly identified in this study as a softening-related gene. PpARF/XYL showed high sequence similarity to a Japanese pear PpARF2, which encodes a protein with multiple enzymatic activities, including XYL and ARF activities (Tateishi et al., 2005). An increase in ARF activity has been observed during peach fruit softening (Brummell et al., 2004), thus, the PpARF/XYL protein may be the ripening-related ARF.

The mRNA accumulation patterns of the softening-related genes were tightly regulated by ethylene, and changes in expression were observed within 12 h after exogenous ethylene treatment was initiated or suspended. These results indicate that ethylene plays important roles in both the induction and maintenance of expression of these softening-related genes in peach. Induction of PpPG mRNA expression was in good accordance with the increase in endoPG enzyme activity that was induced by exogenous ethylene in stony hard peach (Hayama et al., 2006). The time lag of 12–24 h from the start of ethylene treatment to the beginning of the increase in endoPG activity probably reflects the time taken for the transcription and translation of the endoPG gene. It is interesting that the decline in flesh firmness and the accumulation of transcripts of softening-related genes were both quickly repressed when exogenous ethylene treatment was stopped. This suggests that the proteins involved in softening might be quickly inactivated or degraded in response to the cessation of the ethylene signal. This is the first evidence that continuous ethylene perception is required to maintain softening and the accumulation of softening-related mRNAs in peach.

The ethylene concentration is also an important factor in controlling the degree of softening: 0.1 μl l⁻¹ of ethylene was enough to accelerate the softening process, and higher concentrations, up to 100 μl l⁻¹, caused further increases in the rate of softening. In immature pear, 0.1 μl l⁻¹ of ethylene was also sufficient to induce softening (Wang and Hansen, 1970). However, in transgenic cantaloupe melon in which ethylene production was suppressed, 2.5 μl l⁻¹ of ethylene was required for the ethylene-sensitive component of fruit softening, and that was the saturation level of ethylene for flesh softening (Flores et al., 2001). Thus, the sensitivity to ethylene in fruit softening may vary in different fruit species. We also found that, in peach, different softening-related genes showed different sensitivities to ethylene. PpExp3 was the most sensitive of the three genes investigated, and its mRNA was detected at low levels in ‘Manami’ fruit without ethylene treatment (Fig. 1B). The level of PpPG expression was clearly dependent on the concentration of ethylene, which also controlled the rate of softening (Fig. 3). The PpARF/XYL gene appeared to require a higher concentration of ethylene for transcription than the other two genes. The different ethylene sensitivities observed for these softening-related genes might contribute to the ethylene concentration-dependent softening rates of peach fruit.

The other cell wall-related genes, including PpEG4, PpPL1, and PpPL2 showed similar mRNA accumulation patterns in stony hard-flesh ‘Manami’ and melting-flesh ‘Akatsuki’, even though these two cultivars have completely different phenotypes in ethylene production and fruit softening. These results clearly indicated that the transcription of these genes is not sufficient to induce softening in peach, although the proteins encoded by these genes may be involved in softening, and regulated by post-transcriptional processes. It has been shown that mRNAs encoding PLs and EG could be detected in fruit before the climacteric rise in ethylene production (Trainotti et al., 2003, 2006). Therefore, these genes are probably regulated under ethylene-independent pathways. However, it is also possible that they are induced by the very small amount of ethylene that is produced in stony hard peach fruits, as is the case for tomato endoPG (Oeller et al., 1991; Sitrit and Bennett, 1998).

In summary, ethylene influences multiple steps in the initiation and progression of peach fruit softening, and its concentration is also an important factor in regulating the rate of softening. The transcription of softening-related genes such as PpPG, PpARF/XYL, and PpExp3 are also regulated by ethylene, suggesting that peach fruit softening is regulated by ethylene at the transcriptional level, and that constitutive transcription is required for the progression of softening. These findings are useful for understanding the mechanism of peach fruit softening and its relationship with ethylene.

References


