RESEARCH PAPER

Ethylene biosynthesis and perception in apple fruitlet abscission (*Malus domestica* L. Borck)

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Abstract

Abscission was studied in immature apple fruits (cv. Golden Delicious) during the physiological drop. Fruitlet populations, characterized by different abscission potential, were analysed. Non-abscising fruitlets (NAF) were obtained from central flowers borne in clusters where all the lateral flowers had been removed at bloom while abscising fruitlets (AF) were derived from lateral fruitlets of trees sprayed with benzylaminopurine (BAP) at 200 ppm, 17 d after petal fall (APF), when the fruit cross diameter was about 10–12 mm. Fruit shedding, monitored at the end of the June drop, was significantly different in the two populations, being less than 10%, and more than 90%, in NAF and AF, respectively. In AF, fruit drop peaked around 33 d after petal fall (APF) and was preceded by an increase in ethylene around 20 d APF. Transcript analysis was performed from 17–24 d APF, since preliminary experiments pointed out that major changes in expression of abscission related genes occurred within this period. Transcript accumulation of genes involved in ethylene biosynthesis (*MdACS5B* and *MdACO*) and action (*MdERS1*, *MdETR1*, and *MdCTR1*) was studied in the seed, cortex, peduncle, and abscission zone (AZ) of the two fruit populations. *MdACS5B* and *MdACO* transcripts accumulated along the experimental period in AF population, even though at a different magnitude, while ethylene evolution declined after peaking at day three. *MdETR1*, *MdERS1*, and *MdCTR1* expression patterns depended on tissue and/or population. The ERS/ETR ratio was higher in AF than in NAF populations. Overall results pointed out that apple fruitlet drop is preceded by a stimulation of ethylene biosynthesis and a gain in sensitivity to the hormone.

Key words: Abscission, benzylaminopurine, ethylene biosynthesis perception and signal transduction, *Malus domestica*.

Introduction

Many fruit species bear an abundance of flowers producing a surplus of fruits that the tree is unable to support. In anticipation of this, the major fruit species developed an immature fruit (fruitlet) physiological drop as a self-regulatory mechanism. This process is, at least in part, a consequence of the competition among fruits and between fruits and shoots for carbon assimilates.

The self-regulatory mechanism responsible for the immature apple fruit shedding may be magnified by chemicals such as naphthaleneacetic acid (NAA) and its amide and benzylaminopurine (BAP) sprayed within 5–6 weeks after full bloom. The thinning action of bioregulators is quite variable and depends on environmental conditions and varieties. In apple, there are varieties easy to thin and others difficult even though different chemicals or combinations of them are used (Wertheim, 2000). Taking into account the practical importance of thinning in apple and the characteristics of this system, a research programme was designed to elucidate the molecular mechanisms underlying *in planta* fruitlet abscission.

Fruit drop, preceded by a decrease in growth of fruits undergoing shedding, is due to the activation of specific

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Abbreviations: AF, abscising fruitlet; APF, after petal fall; AZ, abscission zone; BAP, benzylaminopurine; CF, central fruitlet; KF, king flower; LF, lateral fruitlet; LBPA, laser-based photoacoustic; NAA, naphthaleneacetic acid; NAF, non-abscising fruitlet; PF, petal fall.

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abscission zones (AZs) (for a complete review see Roberts et al., 2002). It is generally accepted that abscission is a highly regulated developmental process that is both influenced and activated in response to internal cues or environmental conditions (Taylor and Whitelaw, 2001). Nevertheless, the identity of the signal responsible for the activation of the AZ is as yet unknown. Among phytohormones, ethylene enhances abscission in several species and systems (del Campillo and Bennett, 1996; Ruperti et al., 1998; Iannetta et al., 2000; Ueda and Nakamura, 2000; Gonzales-Carranza et al., 2002; Wagstaff et al., 2005). Auxins produced by the seed are thought to desensitize AZ to ethylene and prevent abscission in several systems as well as in apple and cherry (Bangerth, 2000; Drazeta et al., 2004; Else et al., 2004). Considering that seed and/or fruit are involved in determining the shedding signal while the morphogenetic response occurs always at the AZ level, it may be crucial to analyse the system involved in abscission as a whole taking into account concurrently seed, cortex, peduncle, and AZ.

In apple trees, the fruitlet physiological drop is due to the activation of the AZ located at the junction of the peduncle into the twig. In this region four lateral (LF) and one central (CF) fruitlets and the shoot are inserted. The CF comes from the pollinated king flower (KF) that, since it blooms earlier within the cluster, originates a fruitlet larger than the lateral ones. During the physiological drop, the shoot at cluster side, is thought to be a sink in competition with fruitlets for assimilate supply (Bangerth, 2000).

In this paper, data collected from in planta experiments describing the involvement of endogenous ethylene in apple fruitlet abscission and early development are presented. Research was carried out on fruitlet populations characterized by very low and high abscission potential named, respectively NAF (non-abscising fruitlets) and AF (abscising fruitlets). The transcription pattern of genes regulating ethylene biosynthesis and action was studied in seed, cortex, peduncle, and AZ throughout the activation of the shedding process. The possible regulation of ethylene biosynthesis and its role in abscission is discussed, as well as the possible different action of ERS and ETR type receptors.

Materials and methods

Plant material and treatments

Research was carried out in 2003 on 7-year-old apple trees (cv. Golden Delicious/M9) at the experimental farm of the Istituto Agrario Sperimentale, San Michele all’Adige (Trento, Italy). A fruitlet population with high abscission potential (AF) was defined by lateral fruitlets borne on 20 trees sprayed with benylaminopurine (BAP) at 200 ppm (commercial form ‘Brancher-Dirado’) when the average fruit diameter was 10–12 mm (17 d after petal fall, APF). Previous trials demonstrated that, in the same cultivar and at the same concentration, BAP selectively promoted lateral fruitlet abscission that reached values close to 90% (Angeli et al., 2002). A population with very low abscission potential, named non-abscising fruitlets (NAF), was generated by removing, in 20 trees, all the laterals from the cluster at petal fall and leaving, exclusively, the central flower that had been hand-pollinated at bloom with compatible pollen (cv. Stark Red).

Fruitlet shedding and ethylene evolution were registered throughout the physiological drop starting from 2 weeks up to 7 weeks APF in both fruitlet populations. Total abscission was expressed as a percentage per tree of the fruitlet number at petal fall. Abscission dynamics was obtained by plotting abscised fruitlets collected from the ground at 1 d intervals and expressed per tree. Seed, cortex, peduncle, and abscission zone (AZ) samples of each population were collected from fruitlets at 0, 3, 5, and 7 d after the BAP treatment, frozen in liquid nitrogen, and stored at –80 °C for molecular analysis. These sampling dates were chosen since preliminary observations have shown that the largest changes in transcript accumulation of genes under investigation occurred within 7 d.

Ethylene determination

The laser-based photoacoustic (LBPA) detection of ethylene was carried out using a previously described experimental device (Harren et al., 1990a, b; Harren and Reuss, 1997) on the two fruitlet populations. The linear response of the system to the ethylene concentration produced by a 15 fruitlet sample from eight different trees per population was tested by using calibrated gas cylinders (2.5 l h⁻¹ flow rate) and showed a sensitivity of up to 10 parts per trillion (ppt) with a response time of s. Disturbances of the signal by other gases and laser power were simultaneously checked throughout the experiments to normalize the data.

RNA isolation

Total RNA was obtained following the protocol described by Dal Cin et al. (2005). 30 μg of total RNA were treated with 10 U of RQ1 RNase-Free DNase (Promega, Milan, Italy) and 1 U of RNAGuard (RNase INHIBITOR) (Amersham Biosciences, Piscataway, NJ) for 30 min then purified by phenol–chloroform. 1 μg of DNA free total RNA was reverse-transcribed with 200 U of M-MLV Reverse Transcriptase (Promega, Milan, Italy), 1 U of RNAGuard (RNase INHIBITOR) (Amersham Biosciences, Piscataway, NJ) and 2.5 μM oligo-dT₁₂⁻₁₈ as primer at 37 °C for 90 min in a final volume of 20 μl. as described in Sambrook et al. (1989). 1 μl of first-strand cDNA synthesis reaction was used for RT-PCR. Absence of genomic DNA was proved by using primers designed on two exons spanning an intron region of the MdACO; F 5-GAAGCTTTTGGACTTGCTGT-GTGA-3; R 5-CAAATCACGTGGAATCTCAAG-3. The fragment obtained from cDNA is long, around 500 bp, while the genomic one is almost 700 bp. The experiment was performed in 25 μl reactions using 0.025 U μl⁻¹ of AmpliTaq Gold (Applied Biosystems) with a Gene Amp PCR system 9700 (Applied Biosystems, Branchburg, NJ) and 1 μl of cDNA obtained from RNA of samples under investigation as described above. Each cycle (40 in total) consisted of 30 s denaturation at 94 °C, 30 s of annealing at 65 °C, and a 1 min extension at 72 °C; cycles were preceded by a 5 min of denaturation at 94 °C and followed by a final extension period of 7 min at 72 °C. The PCR products obtained were electrophoresed in 2% agarose gels and stained with ethidium bromide. No amplification product coming from genomic DNA was observed under UV light.

MdCTR1 cloning

The MdCTR1 partial cDNA cloning was performed by using a pair of primers designed on the bases of conserved sequence motifs of the CTR1 genes (WNGSDVAV and LEIRPRD) and using 1 μl of cDNA obtained from peduncle, cortex or seed of apple fruitlets as the template. The primers were as follows: F-5-GGMAATGGWMWC-WGAGKTGGCTGT-3; R-5-CAAATCACGTGGAATCTCAAG-3.
Reactions were carried out with the Gene Amp PCR system 9700 (Applied Biosystems, Branchburg, NJ) using 0.025 U µl⁻¹ Taq-polymerase (Amersham Biosciences, Piscataway, NJ) under the following conditions: 5 min at 94 °C followed by 40 cycles of 1 min at 94 °C, 1.5 min at 57 °C, 1 min at 72 °C, and 7 min of final extension at 72 °C.

The amplification product of around 600 bp was subcloned into pGEM-T Easy vector (Promega, Milan, Italy) and nine plasmids from recombinant colonies investigated were sequenced using both SP6 and T7 primers. Plasmids were obtained using the QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) and sequenced by the CRIBI laboratory (Department of Biology, University of Padova) using the ABI Prism BigDye Terminator v3.1 kit (Applied Biosystems, Branchburg, NJ). Sequence comparisons were performed by using BlastX and BlastN algorithms (NCBI, National Centre for Biotechnology Information).

**Gene expression analysis**

Expression of different apple ACS homologues such as *MdACS1* (AB010102), *MdACS2* (U73815), *MdACS3* (U73816), *MdACS5A* (AB034992), and *MdACS5B* (AB034993) was assessed by specific primers designed on non-conserved areas with the support of the online ‘GeneFisher—Software Support for the Detection of Postulated Genes’, according to sequences available in databases and amplifying fragments of around 100 bp. The experiment was performed in 25 µl reactions using 0.025 U µl⁻¹ of AmpliTaq Gold (Applied Biosystems) with a Gene Amp PCR system 9700 (Applied Biosystems, Branchburg, NJ) in cDNA obtained from RNA of samples under investigation and from ripe fruits. For each reaction, a set of different annealing temperatures (from 52 to 66) were tested to verify expression. Each cycle (40 in total) consisted of 30 s denaturation at 94 °C, 30 s of annealing, and a 30 s extension at 72 °C; cycles were preceded by a 5 min of denaturation at 94 °C and followed by a final extension hold of 7 min at 72 °C. PCR products, were electrophoresed on 2% agarose gels and stained with ethidium bromide. Bands of the expected length were gel-extracted, cloned into pGEM-T-easy vector and sequenced as above to confirm the specificity of amplification. The only positive clones were from amplification products obtained using specific primers for *MdACS1* and *MdACS5B*. However, *MdACS1* expression was not detected in the samples under investigation in this work. The primers used are reported in Table 1.

Expression level of *MdACO* (AB030859), *MdETR1* (AF032448), *MdERS1* (AY083169), *MdACS5B* (AB034993), and *MdCTR1* (AY670703) was assessed via quantitative RT-PCR in the facilities of CRIBI (Biology Department, University of Padova) using the SYBR Green RT-PCR master mix kit (PE Applied Biosystems) as described in Cecchetti et al. (2004). For each sample, three replicates were performed in a final volume of 50 µl containing 1 µl of the cDNA (obtained as described above), 15 pmol of specific primers, and 25 µl of 2× SYBR Green PCR Master mix according to the manufacturer’s instructions. The house-keeping gene was ubiquitin (MDU74358) and a further control was represented by primers designed on conserved r18S regions (Rasori et al., 2002). The product specificity of the primers was verified as reported above for *MdACS* genes and controlled by the dissociation protocol at the end of the RT-PCR. The primers used are reported in Table 2.

The experiment was constituted from 10 min at 95 °C, with 40 cycles of 1 min at 72 °C. Each cycle included an initial denaturation at 94 °C for 30 s, an annealing at 62 °C for 30 s and an extension at 72 °C for 30 s. The amount of specific transcripts was calculated following the comparative C₇ method as described by Kenneth and Thomas (2001).

A rough measurement of the ratio between *MdERS1* and *MdETR1* transcripts was done using the results obtained by the comparative C₇ method.

**Table 1. Primer forward (F) and reverse (R) sequences used for the identification of MdACS homologues expressed during abscission in the investigated tissues**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer F</th>
<th>Primer R</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>MdACS1</em></td>
<td>5-CTGGACCTTGGCAATGCAGA-3</td>
<td>5-CGGGAAACCACCTTGTGTA-3</td>
</tr>
<tr>
<td><em>MdACS2</em></td>
<td>5-TGTGATGATGGCAAGTATGTA-3</td>
<td>5-TGGTTTGTGTGAAACAAGTCC-3</td>
</tr>
<tr>
<td><em>MdACS3</em></td>
<td>5-AACAACTCAGAGCTTCACGC-3</td>
<td>5-TCTCCCAACACTAATGATCC-3</td>
</tr>
<tr>
<td><em>MdACS5A</em></td>
<td>5-CTTTGACGAAGAACAAAGAG-3</td>
<td>5-AATGCAATCCCACAGTCTG-3</td>
</tr>
<tr>
<td><em>MdACS5B</em></td>
<td>5-ATGGATGCTCTCGTGCTGAT-3</td>
<td>5-ATAACACATCCCACAGTCC-3</td>
</tr>
<tr>
<td><em>MdACS5B</em></td>
<td>5-CCAACACTCATAAATCACA-3</td>
<td>5-TCTCTCTCATACGGCGATA-3</td>
</tr>
</tbody>
</table>

**Table 2. Primer forward (F) and reverse (R) sequences used for expression analysis of the genes under investigation**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer F</th>
<th>Primer R</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ubiquitin</em></td>
<td>5-CATCCCCCAGACACCACAGA-3</td>
<td>5-ACCACGAGACGGCAACACCAA-3</td>
</tr>
<tr>
<td>r18S</td>
<td>5-GTTACTCTTTAGGACTTCGGGC-3</td>
<td>5-TTCTCTTTAGTTCAGCCTGTC-3</td>
</tr>
<tr>
<td><em>MdACO</em></td>
<td>5-CATCGGAGATGGGACCCAGA-3</td>
<td>5-GCTTTGGAATTTCCAGGCGCA-3</td>
</tr>
<tr>
<td><em>MdACS5B</em></td>
<td>5-CATCGGACCGCTTACAAATG-3</td>
<td>5-GTTGCACTGCTACCTGCAAA-3</td>
</tr>
<tr>
<td><em>MdETR1</em></td>
<td>5-GTGGTCTGTGAAGACGACTG-3</td>
<td>5-TGCAAACCATGCTAGAGCCAT-3</td>
</tr>
<tr>
<td><em>MdERS1</em></td>
<td>5-CATCGGAGATGGGACCCAGA-3</td>
<td>5-GCTTTGGAATTTCCAGGCGCA-3</td>
</tr>
<tr>
<td><em>MdCTR1</em></td>
<td>5-ACAAGATTTTCATGGCCGAAC-3</td>
<td>5-TATGGGACAGTTGGAGGCT-3</td>
</tr>
</tbody>
</table>
A significant difference in ethylene evolution between AF and NAF was observed up to 28 d APF, peaking, in AF, at 20 d APF (3 d after BAP treatment). In NAFs ethylene remained at a basal level along the experimental period. Ethylene burst preceded the abscission peak of 13 d (Fig. 1C).

Expression of genes encoding enzymes involved in ethylene biosynthesis

The expression level of *MdACS5B* appeared to be very low in all tissues. However, transcripts seemed to be more abundant in the cortex than in all the other tissues. The trend was almost constant or slightly declining in NAF. A higher level of transcripts in AF compared with NAF was observed, even though dynamics changed according to tissue (Fig. 2A). In seed, a 2-fold difference between the two populations appeared only at day 7, while in the cortex a difference of the same magnitude was already apparent at day 3, then it declined slightly. In the peduncle, a 2-fold and 3-fold increase occurred at days 3 and 5, respectively. Transcript accumulation at the AZ level in the AF was slightly higher than in NAF and reached a 2-fold increase only at day 7.

*MdACO* appeared to be expressed at a higher level than *MdACS5B* in all tissues of NAF, apart from the cortex. In this population, transcript accumulation showed a constant or a steadily decreasing trend, while a striking accumulation was observed in AF (Fig. 2B). In the latter, the highest level of expression was detected in seed, where the difference between the two populations was already apparent at day 3 (10-fold) and dramatically increased up to day 7 reaching, in AF, an expression level nearly 1000 times higher than in

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**Fig. 1.** Abscission dynamics (A), total abscission (B), and ethylene evolution (C) in non-abscising (NAF) (dotted line and triangles) and abscising (AF) (solid line and squares) fruitlet populations. Total abscission and abscission dynamics were expressed as a percentage of abscission per tree (20 trees per population) and referred to the fruitlets present at petal fall. Ethylene evolution was measured on 15 fruitlet samples collected from AF and NAF populations from 14–38 d APF. Arrows indicate the time of BAP treatment. Values represent means ±SE of 20 independent trees (A, B) and eight fruitlet samples (C).
Fig. 2. *MdACS5B* (A) and *MdACO* (B) transcript accumulation (amount of target) determined by RT-PCR in seed, cortex, peduncle, and abscission zone (AZ) in non-abscising (NAF) (dotted line and triangles) and abscising (AF) (solid line and squares) fruitlet populations for 7 d after the stimulation of abscission with BAP (from 17–24 d after petal fall). Bars indicate the standard deviation of the three PCR replicates.
the NAF population, the value was generally constant or transcripts depicted a situation in which, in all tissues of day 7 compared with NAF. In the AZ of AF, MdACO transcripts peaked at day 5 (4-fold induction) then declined.

**Expression of genes involved in ethylene perception and signal transduction**

The level of expression of *MdETR1* appeared to be quite low in all the tissues investigated (Fig. 3A). In seed of NAF, transcript accumulation showed a declining pattern, while in those of AF the trend was fairly constant. In the cortex, no-significant difference was observed between the fruitlet populations in terms of transcript accumulation, showing a constant pattern up to day 5 and then a slight increase at day 7. In the peduncle and AZ of NAF, transcript accumulation remained nearly constant throughout the experiment, while in the peduncle of AF a sudden decline was observed at days 5 and 7, being more pronounced at day 5. In the AZ of AF, an up-regulation was detected at day 7.

As well as *MdETR1*, the level of expression of *MdERS1* was generally low (Fig. 3B). In all tissues of NAF, transcript accumulation showed a declining or constant pattern, while in AF the gene transcript accumulated in all tissues to a value between two and three times higher than that of NAF by day 5 and 7.

The level of expression of *MdCTR1* was generally lower than that of *MdETR1* (Fig. 4A). In seed and the cortex of the populations a transient slight down-regulation of the gene was observed. In the peduncle, the gene expression remained almost constant in AF, while an up-regulation (2-fold) occurred at days 5 and 7 in NAF. In the AZ of AF, the transcript level significantly increased (three times) by day 7 compared with NAF.

Data about the ratio between the *MdERS1* and *MdETR1* transcripts depicted a situation in which, in all tissues of the NAF population, the value was generally constant or decreasing, while in AF it was generally higher, peaking at day 3 in the cortex and at day 5 in the peduncle and AZ (Fig. 4B).

**Discussion**

**Abscission and ethylene evolution**

Apple fruitlet abscission is a morphogenetic process mainly involving LF which are smaller and at an earlier developmental stage than CFs. As seen in other systems, abscission level depends on many factors among which fruit load appears to be the most important: in fact, these data reconfirmed that, in trees bearing few fruits, abscission was significantly reduced.

Furthermore, by comparing ethylene evolution and fruit drop in the two populations it appears that abscission is related to endogenous ethylene evolution, reconfirming the involvement of the hormone in apple abscission as demonstrated in other fruit species such as peach (Ruperti et al., 1998). Nevertheless, the fact that the ethylene peak precedes by 13 d that of abscission may indicate that fruitlets need a lag phase to gain the ethylene sensitivity at the AZ level and shed from the tree. Moreover, it should be taken into account the possibility that ethylene response may be different according to the tissue or organ.

**Molecular characterization of apple fruitlet shedding**

Molecular results enlighten a situation where seed, cortex, peduncle, and AZ behave both temporally and quantitatively in a different way. Candidate genes involved in ethylene biosynthesis at this fruit developmental stage were identified as *MdACS5B* and *MdACO*. *MdACS5B*, among the members of the gene family so far isolated in apple, was the only one expressed in fruitlets. This gene, already claimed to be involved in the wound response (Sunako et al., 2000) may play an important role in abscission as well. *MdACS2* and *MdACS3* transcripts reported by Rosenfield et al. (1996), were not found in this experiment. This might be assigned to differences in the sequences between ‘Golden Delicious’ and ‘McIntosh’, the cultivar used by the previous authors, or to different fruit developmental stages. Results about *MdACS1* are in accordance with those of Harada et al. (1997) depicting a situation in which this gene is specifically expressed during apple ripening.

The transcript level of *MdACS5B* and *MdACO* was found to be constant or decreasing during early fruitlet development (NAF) while in AF it was, in general, at a higher level. *MdACS5B* appeared to be expressed at a lower level than *MdACO* in AF as observed during peach fruitlet abscission (Bonghi et al., 2000). By comparing *MdACS5B* expression levels in the different tissues and ethylene evolution, it seems likely that the cortex is the main source of ethylene production as this tissue is predominant in terms of fresh weight seed, compared with peduncle, and AZ. The low transcript level of *MdACS5B* found in seed, peduncle, and AZ, and the fact that its increase is postponed compared with that of the cortex may be a consequence rather than a cause of the ethylene burst.

By comparing ethylene evolution and *MdACS5B* transcript accumulation dynamics, it appears that the increase of the hormone level, peaking at day 3 (after BAP treatment) in AF, corresponded only to a slight gene up-regulation. Since, ACS is considered to be a limiting factor in ethylene biosynthesis, it is difficult to explain the large increase in the hormone level simply in terms of *MdACS5B* up-regulation. It could be that the ethylene peak is due mainly to a stabilization and/or activation of the related protein. In fact, *MdACS5B* encodes a protein which displays at the C-terminal a motif similar to ACS2 and ACS6 of *Arabidopsis,*
Fig. 3. *MdETR1* (A), *MdERS1* (B) and transcript accumulation (amount of target) determined by RT-PCR in seed, cortex, peduncle, and abscission zone (AZ) in non-abscising (NAF) (dotted line and triangles) and abscising (AF) (solid line and squares) fruitlet populations for 7 d after the stimulation of abscission with BAP (from 17–24 d after petal fall). Bars indicate the standard deviation of the three PCR replicates.
Fig. 4. MdCTR1 transcript accumulation (amount of target) determined by RT-PCR (A) and MdETR1/MdERS1 transcript ratio (B) in seed, cortex, peduncle, and abscission zone (AZ) in non-abscising (NAF) (dotted line and triangles) and abscising (AF) (solid line and squares) fruitlet populations for 7 d after the stimulation of abscission with BAP (from 17–24 d after petal fall). Bars indicate the standard deviation of the three PCR replicates.
that can undergo phosphorylation by a stress-responsive MAPK, leading to a stabilization of the enzyme (Liu and Zhang, 2005). The involvement of other abscission-related ACS isoforms, as yet unknown, cannot be ruled out.

AF ethylene evolution gradually declined, after peaking at day 3, while MdACO transcripts dramatically continued to increase throughout the experiment at least in the seed and cortex. A similar pattern in terms of a lack of tight correlation between ethylene evolution and transcript accumulation of genes involved in its biosynthesis has been reported in citrus fruits by Katz et al. (2004). This may be due to a post-transcriptional regulation negatively affecting translation, protein activity, and turnover.

MdETR1 transcripts in early developing fruits (NAFs) are more abundant in the peduncle, AZ, and seed than in the cortex, even though the expression always remained at a steady-state level. MdERS1 expression in the different tissues being comparable with that of MdETR1 highlights a situation different, at least in part, from that reported in peach (Rasori et al., 2002) where PpERS1 was expressed at a significantly higher level than PpETR1. An explanation of this may rely on the fact that these genes belong to a multigene family and the level of expression of one member may influence the expression of others (Tieman et al., 2000), so that expression level should be investigated as a whole. At the moment, besides MdETR1 and MdERS1, a LeETR5-like as well as other clones are under characterization. Future research should consider the expression of all these members to find out whether they are involved in abscission as a whole or as a ratio between some specific homologues. Interestingly, the expression level of MdETR1 in the seed and AZ of AFs was at a higher level than that of NAFs (about 2-fold and more than 5-fold, respectively), indicating that, in these tissues, an accumulation of this gene transcript occurs during abscission. Similar results were found in tomato AZ (Lashbrook et al., 1998), while no other information about seeds of abscissing fruitlets is available. As previously found in peach for PpERS1 (Rasori et al., 2002), a possible orthologue of MdERS1, transcripts increased throughout shedding in all tissues of AF indicating a possible role for this receptor in abscission. What is more, the level of MdERS1 transcripts in the AZ at days 5 and 7 may, at least in part, explain the abscission peak occurring later on. An increase of ethylene evolution and/or sensitivity at the AZ level would regulate the expression of genes encoding specific cell wall hydrolases leading to AZ cell separation and to fruitlet shedding (Bonghi et al., 1992, 2000).

Particularly interesting are the results of MdCTR1 expression analysis. An up-regulation in AZ and seed, about 2.5-fold and 1.5-fold, respectively, of AFs and a down-regulation in the peduncle and cortex, about 2-fold and 1.6-fold, respectively, of the same population was found. Similar results were reported in delphinium floret abscission by Kuroda et al. (2004). Some analogies exist between the apple AZ undergoing abscission and the ripening tomato since both are primary targets for ethylene and characterized by an increase in ethylene biosynthesis, a gain in sensitivity to the hormone, as well as an up-regulation of CTR1 (Leclercq et al., 2002; Adams-Phillips et al., 2004). Taking into account that the expression pattern of MdCTR1 and MdETR1 are quite similar, and considering the type of interaction between the receptor and CTR1, the model proposed by Klee (2004) to explain ethylene sensitivity, could be implemented by including the first element of the transductive pathway. Briefly, in the absence of ethylene, receptors are active and suppress ethylene responses while upon ethylene binding, receptors are unable to block ethylene transduction and the hormone response may occur. A decrease in the hormone receptors, in a situation of low and constant ethylene production would lead to an increase in sensitivity, because little ethylene is required to ‘saturate’ the binding sites. The first element of the transductive pathway is CTR1. CTR1, being a negative regulator, when interacting with an empty ethylene receptor blocks the ethylene response. However, in the presence of ethylene, CTR1 does not interact with the receptors and thus the hormone response occurs. Within this dynamic frame the ratio between receptors and CTR1 should be considered since it might be of paramount importance for the ethylene response.

The results and discussions proposed so far are in agreement with the work of Ciardi et al. (2000) showing that an over-expression of NR determined a decrease in ethylene sensitivity while the increase of the overall receptor RNA level occurring in situations of high ethylene evolution (Lashbrook et al., 1998) is believed to be a mechanism to temper the ethylene response, the response to changes in ethylene biosynthesis, and an attempt to maintain homeostasis (Klee, 2004).

In fact, it is clear that the first way that have an ethylene response is to name an increase in the level of the hormone, even without any relevant change in receptors. However, since receptors showed slow-release kinetics for the hormone (O’Malley et al., 2005) an increase in binding sites is necessary to cope with ethylene changes.

An additional way of controlling the ethylene response may rely on the up-regulation of ERS and/or to a modification of the ratio between ERS and ETR type receptors, even without much change in ethylene biosynthesis. In fact, in NAF fruitlets, along with an almost unchanged level of ethylene biosynthesis, a decrease in ERS/ETR occurs. Besides possible differences in terms of kinase activity of the two receptors (Moussatche and Klee, 2004), ERS type receptors, missing the receiver domain, are thought to interact less efficiently with CTR1 (Clark et al., 1998). Moreover, Arabidopsis mutants, characterized by an ETR1 gene encoding a protein missing the receiver domain, displayed a hypersensitive response to low level of ethylene (Qu and Schaller, 2004). Taking into account previous results and
this study’s findings, as well as the fact that NR (the tomato MdiERS1 orthologue) transcripts abundance is related to the amount of the specific protein (Klee, 2004), it can be hypothesized that the ERS/ETR ratio plays a crucial role in abscission by regulating sensitivity to the hormone. An increase of this ratio, although transient as in cortex, peduncle, and AZ of AFs may determine a gain in sensitivity to ethylene.

In conclusion, apple fruitlet abscission, induced by internal and external cues, is preceded by an increase in ethylene evolution which is probably controlled by transcriptional and post-transcriptional mechanisms. The increase in hormone biosynthesis is paralleled by a gain in sensitivity to ethylene that may start a series of events such as senescence-like processes at the level of the cortex and seed where the abscission signal is probably produced. Research is in progress to elucidate the molecular basis of the abscission signal that morphogenetically acts at the AZ level. Tissue ethylene sensitivity appears to be a finely controlled process in which single receptors, overall receptors, receptors ratio, and CTR1 play a crucial role.

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References


