Apple Fruitlet Ethylene Evolution and *MdACO1*, *MdACS5A*, and *MdACS5B* Expression after Application of Naphthaleneacetic Acid, 6-Benzyladenine, Ethephon, or Shading

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Abstract. Abscission of apple (*Malus × domestica* Borkh.) fruitlets is associated with increased ethylene evolution, although the role of ethylene is not clear. Fruitlet ethylene evolution and expression of ethylene-related *MdACO1*, *MdACS5A*, and *MdACS5B* genes were followed in the abscission zone (AZ) and fruit cortex (FC) of king and lateral fruitlets of 'Golden Delicious'/M.9 after spraying with 15 mg·L⁻¹ naphthaleneacetic acid (NAA), 150 mg·L⁻¹ 6-benzyladenine (BA), 500 mg·L⁻¹ ethephon, and after 3 days 96% shading, all applied at a mean 10 mm fruitlet diameter. Lateral fruitlets (LF) were more susceptible to abscission after treatment with chemical thinners or shading compared with king fruitlets (KF) in which only ethephon-treated KF abscised later. On KF, only ethephon increased fruitlet ethylene evolution, *MdACO1* expression in FC and AZ 8 days after treatment (DAT), and abscission of KF 10 DAT, but the other treatments did not. Although ethephon increased *MdACO1* expression in FC of KF 8 DAT, the expression of *MdACS5A* and *MdACS5B* remained unchanged. On LF, all treatments at 8 DAT increased ethylene evolution and *MdACO1* expression in FC and enhanced abscission 10 to 22 DAT. Expression of *MdACS5A* and *MdACS5B* in FC of LF 8 DAT was expressed at a lower level compared with *MdACO1*. In the AZ of both KF and LF, only ethephon increased expression of *MdACO1*, *MdACS5A*, and *MdACS5B*.

Post-bloom application of chemical thinning agents (e.g., NAA, BA, ethephon) is a commonly used measure for the regulation of fruit set in apple (*Malus × domestica* Borkh.) with the goal to improve fruit quality (Link, 2000) and promote annual cropping (Greene, 2002). Ethylene induces (stimulates) organ abscission in numerous plants including apple (Addicott, 1982; Arteca, 1995; Reid, 1985). There is evidence that apple fruitlet abscission may be related to increased ethylene evolution induced by fruit thinning chemicals (McArtney, 2002; Untiedt and Blanke, 2001; Yuan, 2007). Increased ethylene evolution of immature apple fruits was

reported after application of NAA (Zhu et al., 2008), BA (Dal Cin et al., 2005, 2007), and also after application of ethephon (Wittenbach and Bukovac, 1973; Yuan, 2007).

Apple fruitlet abscission may also be promoted by some environmental factors, like warm temperature, cloudy weather, or reduction of photosynthetic active radiation (Wertheim, 2000). Two or three d of artificial shade between 14 and 28 d after full bloom, which is equal as 3 or 4 very cloudy days, can induce fruitlet abscission and reduced fruit set for 83% to 93% (Byers et al., 1991). The effect of artificial shade on fruit set has been confirmed by Zibordi et al. (2009). However, the role of ethylene in the shading-induced fruitlet abscission is unexplained or has not been evaluated.

Furthermore, it is postulated that the fruit position and competition between fruits in cluster influence response of the fruitlet to chemical thinners (Black et al., 1995). Fruitlets without competition in cluster are less susceptible to undergo a abscission process than fruitlets with competition (Dal Cin et al.,

2005). Also, position of the fruitlets in cluster plays a crucial role in abscission process, because Bangerth (2000) proposed that KF dominate over the LF; therefore, response after treatment with chemical thinners of KF compared with LF may be different.

Despite strong evidence that NAA, BA, and ethephon increase ethylene evolution in immature fruits, it is still doubtful if the primary mode of action of chemical thinners is through the increased ethylene synthesis (Untiedt and Blanke, 2001). However, the role of ethylene in fruitlet abscission is also supported by the presence of genes *MdACO1*, *MdACS5A*, and *MdACS5B*, which are involved in the conversion of S-adenosylmethionine to ethylene. Enzymes 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO) are involved in the biosynthetic pathway of ethylene where ACS catalyzes the conversion of S-adenosylmethionine to 1-aminocyclopropane-1-carboxylate (ACC) and in a final step ACO catalyzes the conversion of ACC to ethylene (Yang and Hoffman, 1984). It was thought that ACS was the rate-limiting step in ethylene biosynthesis (Wang et al., 2002), but later Alexander and Grierson (2002) found that ACO is also involved. Genes encoding ACS and ACO are members of multigene families (Wang et al., 2002). In apples, five ACS genes (*MdACS1*, *MdACS2*, *MdACS3*, *MdACS5A*, and *MdACS5B*) (Dal Cin et al., 2005) and four ACO genes (*MdACO1*, *MdACO2*, *MdACO3*, and *MdACO4*) (Wiersma et al., 2007) have been previously isolated and characterized. Based on previous reports, *MdACS1*, *MdACS3*, and *MdACO1* appear to be involved in ethylene formation during fruit ripening (Li et al., 2010; Li and Yuan, 2008; Wiersma et al., 2007). However, increased expression of *MdACS5A*, *MdACS5B*, and *MdACO1* was found before abscission of immature fruit (Dal Cin et al., 2005; Zhu et al., 2008).

The objective of this study was to determine the expression of *MdACO1*, *MdACS5A*, and *MdACS5B* in the AZ and FC after the application of NAA, BA, ethephon, and shading and to relate these findings to ethylene production and the profile of fruitlet abscission. Two groups of fruitlets (KF and LF) with a different potential to abscission were observed in this early (abscission-related) ethylene data study.

Materials and Methods

Plant material, treatments, and sampling.

The experiment was carried out in 2008 in an experimental orchard of the Agricultural Institute of Slovenia at Brdo pri Lukovici (lat. 46°10' N, long. 14°41' E). In the present experiment, 80 10-year-old apple trees, 'Golden Delicious'/M.9, with a minimum of 200 flower clusters were selected and divided into two groups: "A" and "B." From each group, a complete randomized block experiment with eight replications was designed. Trees in experiment "A" were used for ethylene evolution measuring and those in "B" for abscission dynamics and source of samples for genetic

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analyses. In “A,” 10 flower clusters were randomly selected and marked on each tree 2 d after petal fall (DAFP), whereas in “B,” we randomly selected 14 clusters; half were marked as KF (we removed all LF) and the remainder as LF (we removed KF keeping four LF). Thus, we created two types of clusters with different potential for abscission. Treatments in “A” and “B” were identical consisting of: 1) control non-treated; 2) NAA at 15 mg·L⁻¹ (Dirager; L.Gobbi S.r.l., Genoa, Italy); 3) BA at 150 mg·L⁻¹ (MaxCel; Valent BioSciences Corp., Libertyville, IL); 4) ethephon at 500 mg·L⁻¹ (Flordimex 420; Bayer CropScience, Vienna, Austria); and 5) 3-d shading (96% photosynthetic active radiation reduction). At 10-mm KF diameter, trees were sprayed with a low-pressure hand sprayer until runoff. For shading, each tree was surrounded with an engineered frame on which we installed black polypropylene cloth (PP SB 100 ČRN, Filc Mengeš, SLO). The 3 d after the treatment were relatively cold (day temperature 14.9 to 16.7 °C; night temperature 9.4 to 11.3 °C), mostly cloudy, with occasional light rain. Abscission dynamics was followed from 7 to 35 DAPF. The number of KF and LF was determined every second or third day. Fruitlets in clusters, which served for observation of abscission, were also marked to follow each fruitlet from selection to 22 DAT or day of shedding. Fruitlet was estimated as abscised if not presented or light touch induced abscission of fruitlet with a yellow pedicel. Samples for real-time quantitative polymerase chain reaction (qPCR) were collected 1 d before and 8 d after treatment. On each tree, one KF and one LF cluster were randomly selected. Selected fruitlets were cut in half to remove a FC sample (≈100 mg), whereas the AZ sample was collected by cutting a 2-mm segment containing the AZ tissue. Samples were immediately placed into 2-mL collection tubes and frozen in liquid nitrogen. They were stored at -80 °C until RNA extraction.

Ethylene determination. Ethylene evolution was observed on fruitlets attached to the tree on the day of treatments (0 DAT) and 2, 5, and 8 DAT. One KF and one LF cluster from each tree were used to measure ethylene evolution at each sampling date. A selected attached cluster was enclosed in a 245-mL plastic container and maintained on a tree for 3 h. The procedure was similar as described in Burns et al. (1999) with minor modifications. After 3 h incubation, a 1-mL gas sample was collected through the rubber septum in the lid. Then the plastic containers were removed and each cluster was cut and weighed. Ethylene concentration was measured with a gas chromatograph (HP 5890A; Agilent, Santa Clara, CA) with a flame ionization detector.

RNA extraction. Total RNA was extracted from FC and fruit AZ tissue using a RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Starting material was equalized at 50 mg for FC and 25 mg for AZ. Total RNA extraction was performed according to the manufacturer's instructions except 2% (w/v) of polyvinylpyrrolidone (Sigma-Aldrich, Stenheim) was added to the RLT lysis buffer.

Real-time quantitative polymerase chain reaction. Two microliters of total RNA of each sample were treated with DNase I (Invitrogen by Life Technologies, Carlsbad, CA) to remove any contaminating genomic DNA. Absence of genomic DNA was confirmed by using primers spanning an intron in *MdACO* (Dal Cin et al., 2005). First-strand complementary DNA (cDNA) was synthesized by reverse transcribing 2 µL of DNase treated RNA from each sample using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems by Life Technologies). Two microliters of DNase treated RNA were added to 23 µL of RT mix consisting of 2.5 µL 10× RT Buffer; 1 µL dNTP; 2.5 µL Oligo d(T) 16 Primers (Applied Biosystems by Life Technologies); 1.25 µL RNase Inhibitor (Applied Biosystems by Life Technologies); 1.25 µL Multi Scribe reverse transcriptase; and 14.5 µL nuclease free H₂O. Thermal cycling conditions were according to the manufacturer's instructions (Applied Biosystems by Life Technologies). The qPCR reactions were carried out in a 96-well plate format with a total volume of 25 µL, containing 2 µL cDNA, 12.5 µL Power SYBR® Green Master Mix (Applied Biosystems by Life Technologies), 10.2 µL nuclease-free H₂O, and 0.15 µL forward and reverse primers (Table 1). Assays were performed using the ABI 7500 Real-Time PCR System (Applied Biosystems by Life Technologies, Carlsbad, CA) with the following cycling parameters: 95 °C for 10 min and 40 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 40 s. At the end, dissociation curves were examined for the presence of a single polymerase chain reaction product. The efficiencies of the qPCR reactions were calculated for each gene using the relative standard curve with the equation $E (\%) = [(10^{(1/S)} - 1) \times 100]$ (Pfaffl, 2004; Rutledge and Côté, 2003). Expression of each target gene was normalized to a reference gene (actin, CN938023). The efficiencies of amplified genes were comparable (92% to 96%) and relative differences in gene expression were calculated using the 2^{-ΔΔCT} method (Livak and Schmittgen, 2001).

Statistical analysis. Statistical analyses include analysis of variance and Duncan's multiple range tests. Data were analyzed using Statgraphics Centurion XVI (Statpoint Technologies, Warrenton, VA).

Results

Abscission dynamics were expressed as percentage of retained fruitlets on the marked

clusters after counting at 2- to 3-d intervals. Abscission dynamics of KF were non-significant among all treatments except for ethephon (Fig. 1A), which induced intense KF abscission between 8 and 10 DAT. Shedding of LF started after 5 DAT and was most intense on ethephon-treated trees, whereas all other treatments caused more moderate and delayed fruitlet drop (Fig. 1B). At the last measurement (22 DAT), slightly less retained LF was found for BA and ethephon compared with shading, NAA, and control. However, comparing percent retention of LF; control, NAA, BA, and shaded LF followed a similar time course of abscission but 2 weeks later than ethephon treated LF. This is in contrast to KF in which only ethephon treatments induced stronger fruitlet shedding. Final abscission of all LF and KF, regardless of treatments, was 85.7% and 23.5%, respectively (data not shown).

Ethylene evolution was measured before treatment on the day of treatment and 2, 5, and 8 DAT (Fig. 2). No increase in KF ethylene evolution was found at 2 DAT compared with measurements before or among treatments. At 5 DAT, ethylene evolution of control, NAA, BA, or shaded KF slightly decreased but increased in the ethephon treatment. At 8 DAT, we found an additional increase in ethylene evolution in the ethephon-treated KF (approximately threefold compared with 5 DAT). Ethylene evolution from LF 2 DAT was similar to measurement before spraying for all treatments. At 5 DAT, only ethephon increased LF ethylene evolution, 2.9-fold compared with the control. Ethylene evolution between 5 and 8 DAT was further increased for all treatments: 3.1-fold for ethephon, 4.6-fold for shading, whereas 3.3- and 2.2-fold increases were found with NAA and BA, respectively. An increase was also observed in the control, although differences between control and shading, NAA, or BA were non-significant.

Expression of genes *MdACO1*, *MdACS5A*, and *MdACS5B* relative to the untreated control at 8 DAT is presented in Figure 3 and Figure 4. No differences were detected in gene expression in untreated control at 8 DAT and samples before treatment at 0 DAT (data not shown). Compared with the control, differences in expression of *MdACS5A* and *MdACS5B* 8 DAT in FC of KF were significant but very small regardless of treatment (Figs. 3A and C). Ethephon induced expression of *MdACS5A*, *MdACS5B*, and *MdACO1* in AZ of KF, whereas NAA, BA, or shading had very little effect on expression of these genes in AZ tissue

Table 1. Primer forward (F) and reverse (R) sequences used for expression analysis of genes related to ethylene biosynthesis

Gene		Primer 5' → 3'	Accession no.
<i>MdACO1</i>	F	CAGTCGGATGGGACCAGAA	AB030859
<i>MdACO1</i>	R	GCTTGGAAATTCAGGCCAGA	AB030859
<i>MdACS5A</i>	F	GCCAGCCAAGTTTCATAAGC	AB034992
<i>MdACS5A</i>	R	CAACTCTGAAGCCAGGGAAC	AB034992
<i>MdACS5B</i>	F	GAGCAACAGGTTATGGCACA	AB034993
<i>MdACS5B</i>	R	GGTATCGGCGTATGAGGAGA	AB034993
<i>Actin</i>	F	TGACCGAATGAGCAAGGAAATTACT	CN938023
<i>Actin</i>	R	TACTCAGCTTTGGCAATCCACATC	CN938023

(Figs. 3B, D, and F). The increase of *MdACO1* expression in AZ of KF treated with ethephon was 9.2-fold compared with ≈ 6700 -fold in FC of KF (Figs. 3E and F). In contrast, expression of *MdACS5A* and *MdACS5B* in AZ of ethephon-treated KF was 31-fold and 40-fold higher, respectively (Figs. 3B and D). Also in FC of LF, we found some differences in expression of *MdACS5A* and *MdACS5B* between treated samples and untreated control; however, the differences were small and variable (Figs. 4A and C). *MdACO1* expression in NAA-treated FC of LF was 270-fold higher compared with control, whereas BA and shading caused an increase of 1233-fold and 1256-fold, respectively, whereas ethephon increased the *MdACO1* expression 8370-fold compared with the control (Fig. 4E). In AZ of LF, like with AZ of KF, only ethephon caused a 7.7-fold increase of *MdACS5A* expression and a 12-fold increase of *MdACS5B* expression compared with untreated control, whereas other treatments had a small effect on expression of these two genes (Figs. 4B and D). Also, *MdACO1* expression in AZ of LF was increased only with ethephon, whereas other treatments had no significant affect (Fig. 4F).

Discussion

Expression of ethylene-related genes, ethylene evolution, and fruitlet abscission depends of fruitlet type (i.e., KF or LF). In case of KF, high expression of *MdACO1* in FC, ethylene evolution, and abscission was promoted just by ethephon application, whereas other treatments had a small effect on observed parameters. However, in LF, expression of *MdACO1* in FC has been promoted by NAA, BA, ethephon, and shading, and also abscission was increased in all treatments, although with a different abscission profile. Fruitlet abscission has been promoted by ethylene evolution, although the level of ethylene was not directly related to the final fruit set.

Ethephon increased ethylene evolution of KF by 5 DAT and abscission was apparent by 10 DAT, whereas no other treatments induced abscission of KF in this period. We suggest that shedding of ethephon-treated KF was a consequence of higher ethylene evolution. This can be confirmed by gene expression analysis in which ethephon increased the expression of *MdACO1* more than 6700-fold in FC of KF, whereas no significant increases were observed for the other treatments. Despite a high increase of *MdACO1*, the expression of *MdACS5A* and *MdACS5B* in FC of KF remained at the basal level 8 DAT with ethephon. However, for the AZ of KF, next to *MdACO1*, ethephon also increased the expression of *MdACS5A* and *MdACS5B*. It was proposed that increased expression of *MdACO1* is related to the fruitlet abscission (Dal Cin et al., 2009; Zhu et al., 2010), but the role of *MdACS5A* and *MdACS5B* in the abscission process is unclear. It is not clear if expression of *MdACS5A* and *MdACS5B* in AZ indicates the abscission or was this just a further consequence of ethephon action.

Expression of *MdACO1*, *MdACS5A*, and *MdACS5B* has been previously associated

with ethylene production by fruitlets (Li and Yuan, 2008). In our results, it was evident that expression of these genes is more abundant in LF. However, the level of expression of *MdACS5A* and *MdACS5B* in FC of LF was

quite low; therefore, the differences between control and treated samples are small in our experiment. On the contrary, expression of *MdACO1* in FC of LF was very high and the differences among all individual thinning

Fig. 1. Abscission dynamics represented as the percent of retained king fruitlets (KF, **A**) and lateral fruitlets (LF, **B**) observed on selected clusters after the treatment with 15 mg·L⁻¹ NAA, 150 mg·L⁻¹ BA, 500 mg·L⁻¹ ethephon, and 3 days 96% shading. Fruitlet retention was counted in days after treatment (DAT) intervals. Values with bars present means $\pm$ SE, whereas different lower case letters in the column for each DAT indicate significant differences among means drawn on the same vertical order. Means separation was done by Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; BA = 6-benzyladenine.

Fig. 2. Ethylene evolution on king fruitlets (KF, **A**) and lateral fruitlets (LF, **B**) after treatment with 15 mg·L⁻¹ NAA, 150 mg·L⁻¹ BA, 500 mg·L⁻¹ ethephon, and 3 days 96% shading. Ethylene evolution was measured on day of treatment and 2, 5, and 8 days after treatment (DAT). Values with bars present means $\pm$ SE ($n = 8$), whereas different lower case letters in the column for each DAT indicate significant differences among means drawn on the same vertical order. Means separation was done by Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; BA = 6-benzyladenine.

Fig. 3. Real-time quantitative polymerase chain reaction analysis of the expression of genes *MdACS5A*, *MdACS5B*, and *MdACO1* in the king fruitlet (KF) cortex (A, C, E) and KF abscission zone (B, D, F) from 'Golden Delicious' apple trees 8 d after treatment with 15 mg·L⁻¹ NAA, 150 mg·L⁻¹ BA, 500 mg·L⁻¹ ethephon, and 3 days 96% shading. The values of *MdACS5A*, *MdACS5B*, and *MdACO1* transcripts were normalized to actin and calibrated to control. Values with bars present means $\pm$ SE (n = 8), whereas different lower case letters indicate significant differences among means separated by Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; BA = 6-benzyladenine.

treatments compared with the control were significant. This can be an important argument why the *MdACO1* is a suitable indicator of abscission compared with *MdACS5A* and *MdACS5B*, although the importance of the latter two genes cannot be ruled out.

Considering our results, it was evident that expression of *MdACO1* in FC 8 DAT was related with the fruit retention at 22 DAT in case of NAA, BA, ethephon, and shading. Final fruit retention on control trees at harvest was higher than on treated trees; however, the level of expressed *MdACO1* was not directly comparable to the level of finally abscised fruitlets (data not shown). It is likely that the beginning of changes at the molecular level occurs sooner as 8 DAT; however, they are

still present at 8 DAT with significant differences between treatments. The higher expression of *MdACO1* in FC may be an indication of the beginning of the abscission process. In AZ of LF, only ethephon increased the expression of *MdACO1*, *MdACS5A*, and *MdACS5B*. This result is surprising, because *MdACO1* expression was increased in FC of LF of all treatments. It can be postulated that expression of *MdACO1* in FC is not related to the expression in AZ equally for different thinning treatments. Our results on expression of genes related to ethylene metabolism in AZ of KF and LF clearly show that only application of ethephon increased expression of observed genes in both cases. It can be postulated that the abscission process of ethephon-treated

fruitlets is completely dependent on ethylene evolution in contrary to NAA, BA, or shaded fruitlets in which the ethylene probably acts just as a promoter of abscission.

Ethephon-treated KF and LF produced higher amounts of ethylene compared with other treatments 5 DAT and this is reflected in an increase in abscission 10 DAT. Abscission of KF and LF caused by ethephon was faster compared with other treatments but had no stronger effect on final fruit set. Similar results were reported by McCartney (2002) when he compared abscission dynamics between NAA- and ethephon-treated fruitlets. Furthermore, the abscission of shaded LF 8 DAT was in between intensive ethephon LF abscission and almost no NAA, BA, or control LF abscission. Meanwhile, ethylene evolution of shaded LF 5 DAT and 8 DAT was also in between weak ethylene evolution from NAA, BA, or control treatments and high ethylene evolution from ethephon-treated LF. It can be postulated that there is a positive relation between ethylene biosynthesis and abscission dynamics, although it must be stressed that quantity of ethylene evolution 8 DAT did not influence final fruit set. This result suggests that ethylene is a factor that can accelerate the abscission process. Increased ethylene evolution has been reported before abscission as a consequence of NAA (Dennis 2000; Zhu et al., 2008), BA (Dal Cin et al., 2005, 2007), ethephon (Wittenbach and Bukovac, 1973; Yuan, 2007), and shading (Taylor and Whitelaw, 2001; Vandenbussche et al., 2003) applications. However, 2 DAT, no increase in ethylene biosynthesis has been determined on LF, unlike some previous reports (Dal Cin et al., 2007; Zhu et al., 2008). However, it has been previously reported that ethylene evolution is highly temperature-dependent (Olien and Bukovac, 1978; Yuan and Burns, 2004), which means that increasing temperatures resulted in earlier occurrence of peak fruit ethylene production (Curry, 1991), and fruitlets abscised earlier at higher temperature. In our case, temperatures decreased below $\approx 16^{\circ}\text{C}$ for 3 d after treatment with chemical substances. We speculate that this can be the main reason for the delayed fruit ethylene biosynthesis.

To summarize our results, it is evident that LF are more susceptible to abscission induced by chemicals or shading. However, in case of ethephon application, next to LF, also KF undergo abscission, probably because of the increased ethylene evolution, which happened in both types of fruitlets. High ethylene evolution in ethephon-treated fruits, compared with other treatments, could be the reason for followed earlier abscission. However, the quantity of ethylene produced is more related to the abscission course than the final amount of abscised fruitlets. Considering results of expression of genes related to ethylene biosynthesis, it is clear that the level of expression of *MdACO1* in FC is a good indicator for abscission, because expression was increased before abscission of NAA, BA, ethephon, and shaded KF and LF. However, *MdACS5A* and *MdACS5B* were expressed at a much lower level compared

Fig. 4. Real-time quantitative polymerase chain reaction analysis of the expression of genes *MdACS5A*, *MdACS5B*, and *MdACO1* in the lateral fruitlet (LF) cortex (A, C, E) and LF abscission zone (B, D, F) from 'Golden Delicious' apple trees 8 d after treatment with 15 mg·L⁻¹ NAA, 150 mg·L⁻¹ BA, 500 mg·L⁻¹ ethephon, and 3 days 96% shading. The values of *MdACS5A*, *MdACS5B*, and *MdACO1* transcripts were normalized to actin and calibrated to control. Values with bars present means $\pm$ SE (n = 8), whereas different lower case letters indicate significant differences among means separated by Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; BA = 6-benzyladenine.

with *MdACO1*, and also their expression did not correlate with the abscission, except for the ethephon treatment. Regardless of these results, the potential role of *MdACS5A* and *MdACS5B* cannot be avoided. It can be postulated that the mode of action of ethephon is directly through increased ethylene biosynthesis, whereas in case of NAA, BA, and shading, the ethylene acts just as an accelerator of abscission.

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