Aminoethoxyvinylglycine Inhibits Fruit Abscission Induced by Naphthaleneacetic Acid and Associated Relationships with Expression of Genes for Ethylene Biosynthesis, Perception, and Cell Wall Degradation in ‘Delicious’ Apples

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ABSTRACT. Effects of naphthaleneacetic acid (NAA) and aminoethoxyvinylglycine (AVG) on young fruit abscission, leaf and fruit ethylene production, and expression of genes related to ethylene biosynthesis and cell wall degradation were examined in ‘Delicious’ apples (*Malus $\times$ domestica* Borkh.). NAA at 15 mg·L⁻¹ increased fruit abscission and ethylene production of leaves and fruit when applied at the 11-mm stage of fruit development, whereas AVG, an inhibitor of ethylene biosynthesis, at 250 mg·L⁻¹ reduced NAA-induced fruit abscission and ethylene production of leaves and fruit. NAA also increased expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase genes (*MdACS5A* and *MdACS5B*), ACC oxidase gene (*MdACO1*), and ethylene receptor genes (*MdETR1a*, *MdETR1b*, *MdETR2*, *MdERS1*, and *MdERS2*) in fruit cortex and fruit abscission zones. However, AVG reduced NAA-induced expression of these genes except for *MdERS2* in fruit abscission zones. NAA increased expression of the polygalacturonase gene *MdPG2* in fruit abscission zones but not in fruit cortex, whereas AVG reduced NAA-enhanced expression of *MdPG2* in fruit abscission zones. The expression of β -1,4-glucanase gene *MdCell* in fruit abscission zones was decreased by NAA but was unaffected by AVG. Our results suggest that ethylene biosynthesis, ethylene perception, and the *MdPG2* gene are involved in young fruit abscission caused by NAA.

Fruit thinning, which removes excessive fruit from trees at an early stage of fruit development, can improve fruit size, color, and quality; increase return bloom; and reduce alternate bearing of apple trees, thereby increasing growers’ return (Byers, 2003; Childers et al., 1995; Yuan and Greene, 2000a). Because labor is very expensive, fruit thinning is usually conducted by application of chemicals. Compared with hand thinning, chemical thinning also can be done earlier in the season and more effectively increases fruit size, color, and quality (Childers et al., 1995). However, chemical thinning results are extremely variable and very difficult to predict or control because we have an incomplete understanding of the modes of action of chemical thinners (Byers, 2003).

Apple fruitlet abscission after fertilization and during “June drop” has been, at least in part, attributed to competition for carbohydrates among individual fruitlets and between fruitlets and vegetative shoots (Quilan and Preston, 1971; Yuan and Greene, 2000b). Shading or removal of spur and shoot leaves,

which affects leaf photosynthesis and thereby reduces carbohydrates available to young fruit, causes extensive apple fruit abscission (Byers, 2003; Ferree and Palmer, 1982; Yuan and Greene, 2000b). Some researchers reported that the primary mechanism of fruit thinning by chemical thinners such as naphthaleneacetic acid (NAA) and 6-benzylaminopurine (6-BA) is the result of reduced carbohydrates available to developing fruit either by interference with photosynthesis (Stopar et al., 1997; Yuan and Greene, 2000a) or by reduced translocation of metabolites, including photosynthates, from leaves to the fruit (Schneider, 1978).

On the other hand, it has been suggested that chemical thinners such as NAA and 6-BA enhance apple fruitlet abscission through increased ethylene production (Curry, 1991; Dal Cin et al., 2005; McArtney, 2002; Walsh et al., 1979). The pathway of ethylene synthesis has been established in higher plants (Yang and Hoffman, 1984). Ethylene is formed from methionine through S-adenosyl-L-methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (Yang and Hoffman, 1984). The conversion of SAM to 1-aminocyclopropane-1-carboxylate (ACC) and ACC to ethylene are the

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Table 1. Gene-specific primers used for expression analysis of genes related to ethylene biosynthesis, perception, signal transduction, and cell wall degradation.

Gene	Accession no.	Primer left	Primer right
<i>MdActin</i>	CN938023	5'-TGACCGAATGAGCAAGGAAATTACT-3'	5'-TACTCAGCTTTGGCAATCCACATC-3'
<i>MdACS5A</i>	AB034992	5'-GCAATGGTGGTCTTTTCGTATG-3'	5'-TTCGAACGTCTGCTCCTTGA-3'
<i>MdACS5B</i>	AB034993	5'-GAATTTTGTAGTTGGATACCTTCTTT-3'	5'-GAACCAACATCTAAAATCCCATTGT-3'
<i>MdACO1</i>	AB030859	5'-CAGTCGGATGGGACCAGAA-3'	5'-GCTTGGAATTTACAGGCCAGA-3'
<i>MdETR1a</i>	AF032448	5'-TTGGCCTGTGAAGAGCAGT-3'	5'-TGCAAACCATGTAGAGCCAT-3'
<i>MdETR1b</i>	AY821544	5'-GCACTGTGTTTGTCTTGCATT-3'	5'-CGGGACGGCAGGTCAA-3'
<i>MdETR2</i>	DQ847145	5'-GTTGTGACGCGGAAAATGC-3'	5'-AATCCAGATGAAACGGCAGTTAC-3'
<i>MdERS1</i>	AY083169	5'-CAACTAGGGATATGCGAC-3'	5'-CACTGGCATCCAAAGACTTC-3'
<i>MdERS2</i>	AB213028	5'-GCTTGTTAAGGTTGGAAGAAATCTG-3'	5'-CGGCATCGTTGAGTGTACATT-3'
<i>MdCTR1</i>	AY670703	5'-ACAAGATTTTCATGCCGAAC-3'	5'-TATGGACAAGTTTGGAGGCT-3'
<i>MdPG2</i>	AB210897	5'-CGTTTCAGCCGACAAGTTG-3'	5'-TACGAGTGAGGAGGAGTAGATGGA-3'
<i>MdCell</i>	AY350734	5'-ACCAGAACGATGGATTTCAGAT-3'	5'-GTACGTTGCAGGCTCCGAAT-3'

rate-limiting steps in ethylene biosynthesis and are catalyzed by ACC synthase (ACS) and ACC oxidase (ACO), respectively (Alexander and Grierson, 2002; Wang et al., 2002). Genes encoding ACS and ACO are members of multigene families, and their expression is differentially regulated by a variety of biotic and abiotic factors (Kende, 1993; Wang et al., 2002). In apples, five ACS genes, *MdACS1*, *MdACS2*, *MdACS3*, *MdACS5A*, and *MdACS5B*, and one ACO gene, *MdACO1*, have been isolated and characterized (Dal Cin et al., 2005; Li and Yuan, 2008). *MdACS1* and *MdACO1* are related to the burst of fruit ethylene production during fruit ripening in apples, whereas *MdACS5B* and *MdACO1* are associated with young fruit ethylene production (Dal Cin et al., 2005; Li and Yuan, 2008).

Aminoethoxyvinylglycine is a potent inhibitor of ethylene biosynthesis through inhibiting ACS enzyme activity (Boller et al., 1979). Application of aminoethoxyvinylglycine (AVG) inhibits fruit ethylene production and expression of *MdACS1*, *MdACS5A*, and *MdACO1* and delays fruit ripening and preharvest fruit abscission in apples (Li and Yuan, 2008; Schupp and Greene, 2004; Yuan and Carbaugh, 2007; Yuan and Li, 2008).

After synthesis, ethylene is perceived by a family of membrane-localized receptors. In *Arabidopsis thaliana* (L.) Heynh], there are five known ethylene receptors, *ETR1*, *ETR2*, *ERS1*, *ERS2*, and *EIN4* (Wang et al., 2002). These receptors seem to undergo conformational changes on the binding of ethylene and then interact with the Raf-like serine/threonine kinase CTR1, a negative regulator of ethylene signal transduction. The signal then passes down a partially elucidated cascade that ultimately controls a myriad of ethylene-associated plant growth and development processes (Klee, 2004; Wang et al., 2002). In apples, it has been reported that ethylene receptor genes, *MdETR1*, *MdETR2*, *MdERS1*, and *MdERS2*, and ethylene signal transduction gene, *MdCTR1*, are involved in fruit ripening and young fruit abscission (Dal Cin et al., 2005; Li and Yuan, 2008).

It has been reported that concomitant with increased ethylene production is increased expression of genes and activity of enzymes associated with cell wall degradation such as β -1,4-glucanase (cellulase or EG) and polygalacturonase (PG) (Bonghi et al., 2000; Roberts et al., 2002), which causes the middle lamellae of abscission zone cells to dissolve and, ultimately, the organ to abscise. Other genes such as pathogenesis-related genes and those involved in secondary metabolism and signal transduction are also enhanced during the abscission process (Roberts et al., 2002).

Fig. 1. Effects of NAA and AVG on (A) fruit abscission pattern and (B) total fruit abscission in 'Delicious' apples in 2007. Data are means $\pm$ SE (n = 4). Different letters indicate significant differences among means according to Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; AVG = aminoethoxyvinylglycine.

Fig. 2. Effects of NAA and AVG on (A) leaf ethylene production and (B) fruit ethylene production in 'Delicious' apples in 2007. Data are means $\pm$ SE ($n = 4$). Different letters indicate significant differences among means according to Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; AVG = aminoethoxyvinylglycine.

The purpose of this study was to evaluate whether ethylene biosynthesis, ethylene perception, and cell wall degradation were involved in young fruit abscission caused by the chemical thinner NAA in 'Delicious' apples.

Materials and Methods

PLANT MATERIAL AND TREATMENTS. Sixteen 13-year-old 'Delicious' apple trees grafted on 'M.111' rootstock were selected in an orchard located at Alson H. Smith, Jr. Agricultural Research and Extension Center, Winchester, VA, and blocked into four groups of four trees each. Apple trees had an average of 2.5 m in canopy height and 2.7 m in canopy diameter. A randomized complete block design with four replications was used. One tree from each block received one of the four treatments on 14 May 2007 when fruit size was ≈ 11 mm in diameter. Treatments consisted of: 1) water, which served as control; 2) NAA (Fruitone N; AMVAC, Newport Beach, CA) at $15 \text{ mg} \cdot \text{L}^{-1}$; 3) AVG (ReTain; Valent BioSciences, Libertyville, IL) at $250 \text{ mg} \cdot \text{L}^{-1}$; and 4) NAA at $15 \text{ mg} \cdot \text{L}^{-1}$ + AVG at $250 \text{ mg} \cdot \text{L}^{-1}$. All spray solutions contained Silwet-77 silicone surfactant (Loveland Industries, Loveland, CO) at 0.125% to improve

dispersion. The surfactant had no effect on fruit and leaf ethylene production. Solutions were applied to the canopy with a low-pressure hand-wand sprayer until runoff. NAA was applied ≈ 1 h after application of AVG. Leaves and young fruit were dry when NAA was applied. Average daily high and low temperature in the first 3 d after treatment was $\approx 26/13^\circ \text{C}$.

In 'Golden Delicious' apples, we found that NAA, applied at the 11-mm stage of fruit development, markedly increased young fruit ethylene production and enhanced expression of genes related to ethylene biosynthesis, perception, and cell wall degradation 1, 3, and 5 d after treatment (H. Zhu, E. Beers, and R. Yuan, unpublished data). In this study, both leaf and young fruit samples were collected from each 'Delicious' apple tree of three replicate blocks 4 d after treatment (≈ 26 d after full bloom). The fruit samples were immediately separated into cortex and fruit abscission zones. Fruit abscission zones were collected by cutting 1 mm at each side of the abscission fracture plane. Promptly after separation of fruit, all samples were frozen in liquid nitrogen and stored at -80°C for extraction of RNA.

DETERMINATION OF FRUIT ABSCISSION AND ETHYLENE PRODUCTION OF FRUIT AND LEAVES. To determine fruit abscission rate, two limbs on each tree were tagged. Fruits on tagged limbs were counted just before treatment, and then fruit remaining on tagged limbs were counted every 2 or 3 d. To determine ethylene production of fruit and leaves, 15 fruit and 20 leaves were collected from each tree 2 and 4 d after treatment, enclosed in 100- and 1000-mL containers, respectively, and incubated for 3 h. One milliliter of gas sample was withdrawn from the sealed container through the rubber septum affixed to lid, and ethylene concentration was measured with a gas chromatograph equipped with an activated alumina column and FID detector (model 3700; Varian, Palo Alto, CA).

TOTAL RNA EXTRACTION AND REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION. Total RNA was extracted from fruit abscission zones and fruit cortex as described by Li and Yuan (2008). DNA was removed from each RNA sample using the TURBO DNA-free™ Kit (Ambion, Austin, TX). Reverse transcriptase–polymerase chain reaction was performed using primers that span an intron in *MdACO* to confirm that each RNA sample was free of genomic DNA contamination (Li and Yuan, 2008).

One microgram of total RNA was used to synthesize cDNA in a 20 μL reaction volume using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time quantitative polymerase chain reaction (PCR) was performed using the Power SYBR Green PCR Master Mix Kit (Applied Biosystems) on an Applied Biosystems 7500 Real-Time PCR System according to the manufacturer's instructions. Gene-specific primers were designed for nonconserved areas using Primer Expression 3.0 software (Applied Biosystems) and synthesized by Integrated DNA Technologies (Coralville, IA). The primer sequences are listed in Table 1. Real-time samples were run in triplicate and the reaction volumes were 25 μL . Dissociation curves were generated to determine the specificity of the amplification reactions. In addition, the amplified products were sequenced as described by Li and Yuan (2008). After validation tests, normalization to actin was performed using the $\Delta\Delta C_T$ method (Applied Biosystems, 2005).

Fig. 3. Real-time quantitative polymerase chain reaction analysis of the expression of *MdACS5A*, *MdACS5B*, and *MdACO1* in (A, C, and E) fruit abscission zones and (B, D, and F) fruit cortex from 'Delicious' apple trees 4 d after application of NAA and AVG. The levels of *MdACS5A*, *MdACS5B*, and *MdACO1* transcripts were normalized using actin. Data are means $\pm$ SE ($n = 3$). The values of *MdACS5A*, *MdACS5B*, and *MdACO1* in fruit abscission zones and fruit cortex from water-treated control 'Delicious' apple trees were arbitrarily set to 1. Different letters indicate significant differences among means according to Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; AVG = aminoethoxyvinylglycine.

STATISTICAL ANALYSES. Statistical analyses included analysis of variance and Duncan's multiple range test. SAS software for PC (SAS Institute, Cary, NC) was used to analyze the results.

Results

EFFECT OF NAPHTHALENEACETIC ACID AND AMINOETHOXYVINYLGLYCINE ON FRUIT ABSCISSION AND ETHYLENE PRODUCTION OF FRUIT AND LEAVES. NAA at $15 \text{ mg}\cdot\text{L}^{-1}$ effectively increased fruit abscission, whereas AVG at $250 \text{ mg}\cdot\text{L}^{-1}$ reduced NAA-enhanced fruit abscission in 'Delicious' apples (Fig. 1A–B). The rate of NAA-induced fruit abscission peaked ≈ 14 d after treatment. Compared with the water-treated control, ethylene production of NAA-treated leaves increased ≈ 9 -fold 2 d after treatment (Fig. 2A). However, there was no difference in leaf ethylene production between the control and NAA 4 d after treatment. AVG reduced NAA-induced leaf ethylene production 2 d after treatment. Fruit ethylene production was also stimulated by NAA 2 and 4 d after treatment (Fig. 2B). As was observed for leaves, there was no difference in fruit ethylene

production between control and the AVG-only treatment. AVG virtually eliminated NAA-induced fruit ethylene production.

EFFECT OF NAPHTHALENEACETIC ACID AND AMINOETHOXYVINYLGLYCINE ON EXPRESSION OF GENES ENCODING ENZYMES INVOLVED IN ETHYLENE BIOSYNTHESIS. Very low or no expression of *MdACS1*, *MdACS2*, and *MdACS3* was detected in fruit abscission zones and fruit cortex (data not shown). The expression of *MdACS5A*, *MdACS5B*, and *MdACO1* in fruit cortex and fruit abscission zones was increased by NAA application (Fig. 3A–F). The cortex of fruit from trees treated with AVG alone had lower levels of *MdACS5B* transcripts than that from water-treated control trees. However, there was no significant difference in the levels of *MdACS5B* in fruit abscission zones between AVG alone and the water-treated control. There was no difference in the levels of *MdACS5A* and *MdACO1* transcripts in fruit cortex and fruit abscission zones between the water-treated control and AVG alone either. NAA-induced expression of *MdACS5A*, *MdACS5B*, and *MdACO1* in fruit cortex and fruit abscission zones was decreased by AVG.

EFFECT OF NAPHTHALENEACETIC ACID AND AMINOETHOXYVINYLGLYCINE ON EXPRESSION OF GENES ENCODING ETHYLENE RECEPTORS AND ETHYLENE SIGNAL TRANSDUCTION KINASE *CTR1*. The levels of *MdETR1a*, *MdETR1b*, and *MdETR2* transcripts in fruit

abscission zones and fruit cortex were increased by NAA (Fig. 4). Expression of *MdETR1a* and *MdETR1b* in fruit cortex and fruit abscission zones was unaffected by AVG alone. AVG alone increased expression of *MdETR2* in fruit abscission zones but not in fruit cortex. NAA-induced expression of *MdETR1a*, *MdETR1b*, and *MdETR2* in fruit abscission zones and fruit cortex was reduced by AVG.

The levels of *MdERS1*, *MdERS2*, and *MdCTR1* transcripts in fruit abscission zones and fruit cortex were increased by NAA (Fig. 5). AVG alone increased expression of *MdERS2* in fruit abscission zones, but it had no effect on expression of *MdERS2* in fruit cortex. AVG alone did not affect expression of *MdERS1* and *MdCTR1* in fruit abscission zones and fruit cortex. NAA-induced expression of *MdERS1*, *MdERS2*, and *MdCTR1* in fruit cortex was reduced by AVG. AVG reduced NAA-induced expression of *MdERS1* but not of *MdERS2* or *MdCTR1* in fruit abscission zones.

EFFECT OF NAPHTHALENEACETIC ACID AND AMINOETHOXYVINYLGLYCINE ON EXPRESSION OF GENES ENCODING ENZYMES INVOLVED IN CELL WALL DEGRADATION IN 'DELICIOUS' APPLES. The expression of *MdCell1* in fruit abscission zones and fruit

Fig. 4. Real-time quantitative polymerase chain reaction analysis of expression of *MdETR1a*, *MdETR1b*, and *MdETR2* in (A, C, and E) fruit abscission zones and (B, D, and F) fruit cortex from 'Delicious' apple trees 4 d after application of NAA and AVG. The levels of *MdETR1a*, *MdETR1b*, and *MdETR2* transcripts were normalized using actin. Data are means $\pm$ SE ($n = 3$). The values of *MdETR1a*, *MdETR1b*, and *MdETR2* in fruit abscission zones and fruit cortex from water-treated control 'Delicious' apple trees were arbitrarily set to 1. Different letters indicate significant differences among means according to Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; AVG = aminoethoxyvinylglycine.

cortex was decreased by NAA but was unaffected by AVG (Fig. 6A–B). Expression of *MdPG1* was not detected in fruit abscission zones and fruit cortex (data not shown). *MdPG2* expression in fruit abscission zones was increased by NAA but was unaffected by AVG alone (Fig. 6C). AVG reduced NAA-induced expression of *MdPG2* in fruit abscission zones. Expression of *MdPG2* in fruit cortex was unaffected by NAA but reduced by AVG and NAA + AVG.

Discussion

In this study, NAA increased ethylene production of young fruit and leaves and increased young fruit abscission in 'Delicious' apples. This is consistent with previous reports that ethylene production of young fruit and leaves increased rapidly in response to postbloom thinning spray of NAA (Curry, 1991; Dal Cin et al., 2005; McArtney, 2002; Walsh et al., 1979). We expanded on this observation and showed that NAA-induced ethylene production of young fruit and leaves and young fruit

abscission were reduced by AVG, a well-known inhibitor of ACS activity (Boller et al., 1979). These results suggest that NAA-induced young fruit abscission is associated with ethylene biosynthesis in apples.

It has been suggested that regulation of ethylene biosynthesis by various stresses and endogenous signals is mainly through the differential expression of ACS and ACO genes (Kende, 1993). Auxin stimulates ethylene production by enhancing ACS expression in various plant species (Abel and Theologis, 1996; Li and Yuan, 2008). Our results showed that expression of *MdACS5A*, *MdACS5B*, and *MdACO1* increased significantly in NAA-treated fruit cortex and fruit abscission zones, whereas very low or no expression of *MdACS1*, *MdACS2*, and *MdACS3* was detected. On the other hand, AVG effectively reduced NAA-enhanced expression of *MdACS5A*, *MdACS5B*, and *MdACO1*. These results suggest that *MdACS5A*, *MdACS5B*, and *MdACO1* but not *MdACS1*, *MdACS2*, or *MdACS3* are related to NAA-induced ethylene production of young fruit and young fruit abscission in 'Delicious' apples.

Our results showed that expression of ethylene receptor genes *MdETR1a*, *MdETR1b*, *MdETR2*, and *MdERS1* in fruit abscission zones and fruit cortex and *MdERS2* in fruit cortex was increased by NAA, but AVG reduced NAA-induced expression of these genes, suggesting that NAA-induced expression of these receptors may be dependent on increased ethylene production. Other investigators have also suggested that the increase in the levels of overall receptor mRNA during fruit abscission may be a natural response to increased ethylene biosynthesis (Dal Cin et al., 2005; Kevany et al., 2007; Klee, 2004). Moreover, that the application of AVG reduced NAA-induced expression of *MdETR1a*, *MdETR1b*, *MdETR2*, and *MdERS1* genes in fruit abscission zones and NAA-induced fruit abscission is suggestive of a role for these ethylene receptors in NAA-induced young fruit abscission in 'Delicious' apples. A similar correlation between abscission and increased expression of ethylene receptor genes in abscission zones has been reported in flowers of tomato (*Solanum lycopersicum* L.) (Lashbrook et al., 1998; Whitelaw et al., 2002) and young fruit of apples (Dal Cin et al., 2005). Also consistent with our finding is the observation that reduction of *LeETR1* transcript levels by antisense *LeETR1* delayed the abscission of flowers and leaves in tomato (Whitelaw et al., 2002). However, the observed correlation seems contradictory to the model that ethylene receptors negatively

Fig. 5. Real-time quantitative polymerase chain reaction analysis of expression of *MdERS1*, *MdERS2*, and *MdCTR1* in (A, C, and E) fruit abscission zones and (B, D, and F) fruit cortex from 'Delicious' apple trees 4 d after application of NAA and AVG. The levels of *MdERS1*, *MdERS2*, and *MdCTR1* transcripts were normalized using actin. Data are means $\pm$ SE ($n = 3$). The values of *MdERS1*, *MdERS2*, and *MdCTR1* in fruit abscission zones and fruit cortex from water-treated control 'Delicious' apple trees were arbitrarily set to 1. Different letters indicate significant differences among means according to Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; AVG = aminoethoxyvinylglycine.

regulate ethylene responses and there is an inverse relationship between receptor levels and ethylene sensitivity of a tissue (Hua and Meyerowitz, 1998; Klee, 2004). Further work will be necessary to determine the relationship between the levels of ethylene receptor proteins in abscission zones and fruit abscission.

It has been well documented that an increase in PG and cellulase activities is usually associated with fruit abscission (Bonghi et al., 2000; Li and Yuan, 2008). No expression of *MdPG1* was detected in the abscission zones of young 'Delicious' apple fruit regardless of treatment (data not shown), suggesting that *MdPG1* is not related to young fruit abscission in apples. Similarly, Li and Yuan (2008) reported that *MdPG1* is not involved in mature fruit abscission in apples. On the other hand, NAA increased *MdPG2* expression in fruit abscission zones, but the increase was reduced by AVG. These results suggest that *MdPG2* is related to NAA-induced young fruit abscission. Our results also showed that expression *MdCell1*, which encodes cellulase, was significantly decreased by NAA but unaffected by AVG in fruit abscission zones. This indicates

that *MdCell1* is unlikely involved in young fruit abscission induced by NAA.

Carbohydrates and fruit ethylene production play a critical role in young fruit abscission in apples (Byers, 2003; Curry, 1991; McArtney, 2002; Stopar et al., 1997; Walsh et al., 1979; Yuan and Greene, 2000a). However, the relationship between carbohydrates and fruit ethylene production is not clear. Recent studies have revealed that sugars not only provide carbon and energy, but also play a pivotal role as signaling molecules in plants that integrate external environment conditions with intrinsic developmental programs modulated by multiple plant hormones (Rolland et al., 2006; Thimm et al., 2004). DNA microarray analysis showed that shading or low sugar concentrations upregulated genes involved in biosynthesis and signaling of abscisic acid (ABA) and ethylene in arabidopsis plants (Cheng et al., 2002; Kim and Arnim, 2006; Thimm et al., 2004), whereas application of glucose downregulated genes upregulated by both shading and ABA (Kim and Arnim, 2006). It also has been reported that defoliation- or shading-induced young fruit abscission was preceded by an increase in the levels of ABA and ACC in citrus [*Citrus unshiu* (Mak.) Marc.] (Gomez-Cadenas et al., 2000; Iglesias et al., 2006). Therefore, it is possible that NAA not only directly increases fruit ethylene production by increasing expression of

MdACS5A and *MdACS5B*, but also indirectly increases fruit ethylene production through increasing biosynthesis and signaling of ABA and ethylene by reducing photosynthesis and carbohydrate levels. More research work will be necessary to determine the relationship between carbohydrate shortage and young fruit ethylene production.

Unlike the positive effect NAA has on abscission of young apple fruit, NAA reduces mature apple fruit abscission although it increases fruit ethylene production and fruit softening (Li and Yuan, 2008). Using real-time quantitative PCR, Li and Yuan (2008) found that NAA reduced mature apple fruit abscission by inhibiting expression of *MdPG2* in fruit abscission zones, increased mature fruit ethylene production by increasing expression of *MdACS1* and *MdACO1*, and enhanced mature fruit softening by increasing expression of *MdPG1* in fruit cortex. Further efforts are needed to determine how NAA increases expression of *MdPG2* in abscission zones of young fruit but inhibits its expression in mature fruit abscission zones.

In summary, our results showed that NAA increased young apple fruit abscission through increasing expression of

Fig. 6. Real-time quantitative polymerase chain reaction analysis of expression of *MdCel1* and *MdPG2* in (A and C) fruit abscission zones and (B and D) fruit cortex from 'Delicious' apple trees 4 d after application of NAA and AVG. The levels of *MdCel1* and *MdPG2* transcripts were normalized using actin. Data are means $\pm$ SE (n = 3). The values of *MdCel1* and *MdPG2* in fruit abscission zones and fruit cortex from water-treated control 'Delicious' apple trees were arbitrarily set to 1. Different letters indicate significant differences among means according to Duncan's multiple range test ($P < 0.05$). NAA = naphthaleneacetic acid; AVG = aminoethoxyvinylglycine.

MdACS5A, *MdACS5B*, and *MdACO1*; fruit ethylene production; and expression of *MdETR1a*, *MdETR1b*, *MdETR2*, *MdERS1*, *MdERS2*, and *MdPG2* in fruit abscission zones. In contrast, AVG reduced NAA-induced young apple fruit abscission by inhibiting fruit ethylene production and expression of *MdACS5A*, *MdACS5B*, *MdACO1*, *MdETR1a*, *MdETR1b*, *MdETR2*, *MdERS1*, and *MdPG2* in fruit abscission zones.

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