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Effects of Preharvest Aminoethoxyvinylglycine (AVG) Treatment on Fruit Ripening, Core Browning and Related Gene Expression in 'Huangguan' Pear (*Pyrus bretschneideri* Rehd.)

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Abstract: 'Huangguan' pear (*Pyrus bretschneideri* Rehd. cv. Huangguan) is a widely planted cultivar in China. However, it is susceptible to core browning after harvest. In this study, aminoethoxyvinylglycine (AVG) was applied at 200 mg L^{-1} one and two weeks prior to harvest, and its effects on fruit quality, ripening and core browning were investigated during fruit storage at ambient temperature (25 ± 1 °C). The results showed that there was higher firmness, soluble solids content (SSC) and titratable acid (TA) content, but a lower ethylene production rate and core browning index in AVG-treated fruit than in control (water). Compared with the control fruit, AVG treatment decreased the malondialdehyde (MDA) content and polyphenol oxidase (PPO) activity, delayed the peak of chlorogenic acid (CGA) content in the core tissue, and significantly inhibited the expression of genes such as ACC synthase (*PbACS2*, *PbACS3a*, *PbACS5a* and *PbASC5b*), ACC oxidase (*PbACO1* and *PbACO2*), ethylene receptors (*PbETR2* and *PbERS1*), ethylene response factor (*PbERF1*), phenylalanine ammonia lyase (*PbPAL1*), cinnamate 4-hydroxylase (*PbC4H4*), 4-hydroxycinnamoyl-CoA ligase (*Pb4CL2*), hydroxycinnamoyl- CoA shikimate hydroxycinnamoyl transferase (*PbHCT1* and *PbHCT3*), and polyphenol oxidase (*PbPPO1* and *PbPPO5*), as well as phospholipase D (*PbPLD*) and lipoxygenase (*PbLOX1* and *PbLOX5*). Thus, these results suggested that the reduction in core browning by preharvest application of AVG might be due to an inhibitory effect on the expression of genes associated with ethylene biosynthesis and signaling pathways, CGA biosynthesis, PPO and cell membrane degradation in 'Huangguan' pear.

Keywords: pear; cell membrane; ethylene; phenolics; browning

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1. Introduction

Ethylene plays an important role in regulating fruit ripening and senescence. 1-Aminocyclopropane-1-carboxylate (ACC) synthase (ACS) and ACC oxidase (ACO) are key enzymes in the ethylene biosynthesis pathway [1]. Aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, can prevent the transformation of *S*-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid, and thus decrease ACS activity and inhibit ethylene production [2], delay fruit ripening, especially in climacteric fruit [3–12], and reduce the internal browning [13–17].

The ethylene signaling pathway has been studied over the past few decades. Ethylene is perceived as extracellular signal by its receptors, including ETR1 (Ethylene Response 1), ETR2, ERS1 (Ethylene Response Sensor 1), ERS2, and EIN4 (Ethylene Insensitive 4), which remove the inhibitory effect of CTR1 (Constitutive Triple Response 1) on EIN2. EIN2 further activates EIN3/EIL1 (EIN3-like1), which induce the expression of secondary transcription factors in the ethylene signaling pathway, such as ERFs (ethylene response factors) [18–21]. ERF is considered to be the ultimate response factor in the ethylene signaling pathway,

and it can bind directly to target gene promoters to regulate various biological processes relating to fruit ripening and senescence [22,23], including genes associated with ethylene biosynthesis [24] and anthocyanin biosynthesis [25,26].

Ethylene blocking measures, such as lysophosphatidylethanolamine and 1-methylcyclopropene, can delay ripening and senescence of the climacteric fruit, in the way of maintaining membrane integrity [27], regulating hormone production [28], inhibiting the activation of ethylene biosynthesis and tissue browning related genes [29].

Fruit browning is closely related to phenolic metabolism and polyphenol oxidase (PPO) [30–33]. Chlorogenic acid (CGA) is a primary phenolic compound in pears and is generally recognized as the key substrate that results in enzymatic browning catalyzed by PPO [30,31,34,35]. Phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H4), 4-hydroxycinnamoyl-CoA ligase (4CL), and hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase (HCT) are the main enzymes for the biosynthesis pathway of CGA [35]. Moreover, fruit browning is also closely related to the breakdown of the cell membrane, which is associated with the activities of phospholipase D (PLD) and lipoxygenase (LOX) [36–41].

'Huangguan' pear (*Pyrus bretschneideri* Rehd. cv. Huangguan) is a widely planted cultivar in China, and is well known due to its regular fruit shape and fine quality at harvest. However, there is a high incidence of core browning in 'Huangguan' pears for 10 days or so of storage at room temperature [42], and the mechanism of core browning and the regulation role of AVG is still poorly understood [16]. The objective of this study was to investigate the effect of AVG on fruit quality and core browning, and further to reveal the molecular mechanism of ethylene involved in the development of core browning in 'Huangguan' pear.

2. Materials and Methods

2.1. Materials and Treatments

The experiment was performed at a commercial 'Huangguan' pear (*Pyrus bretschneideri* Rehd. cv. Huangguan) orchard (20-year-old) in Zhao County, Hebei Province, China. It was arranged as a completely random design with three single-tree replicates per treatment, in which pear trees with uniform size and fruit load were used, planting with 3 m × 2 m rowing space. ReTain® (Valent Bioscience, Libertyville, IL, USA), a commercial product containing 15% (*w*/*w*) AVG, was applied at one (4 August 2016) and two weeks (28 July 2016) prior to harvest (11 August 2016). Fifteen liters of aqueous solution of AVG containing 0.02% (*v*/*v*) Tween-20 was sprayed to each tree, and the water was used as control. According to a preliminary experiment, the AVG concentration with 200 mg L^{-1} was the most effective (Supplementary Figure S1). Fruits were transported to the laboratory within 2 h after harvest, and those with a similar weight (average weight per fruit: 271.40 ± 4.80 g) and without mechanical damage were selected and transferred to commercial fruit cartons. Then, the cartons with 30 fruits in each were stored at ambient temperature (25 ± 1 °C and R.H. of 90%).

Fruit quality parameters and the ethylene production rate were determined on a whole fruit basis. After measuring the core browning index, the core tissue without seeds was immediately frozen by liquid nitrogen and kept at −80 °C until further study.

2.2. Determination of Fruit Quality

Flesh firmness was measured by a fruit firmness meter (Model GY-J, Top Instruments, Hangzhou, China) at two opposite points on the equatorial part of each fruit after skin removal. The soluble solids content (SSC) was determined by a digital refractometer (Model PAL-1, Atago, Tokyo, Japan). The titratable acid (TA) content was determined using a base burette by titration with 0.01 mol L^{-1} NaOH up to a pH of 8.1 and was expressed as percent (%) of malic acid. Three replications each treatment, 5 fruits each replication.

2.3. Estimation of the Core Browning Index

A transverse section was cut through the fruit and the level of core browning was scored as follows: 0–3 corresponded to no core browning, <25% core browning, 25–50% core browning, and >50% core browning, respectively. The core browning index was calculated using the following formula [43]:

$$\text{Core browning index} = \sum (\text{Score level} \times \text{number of fruit at the level}) / [3 \times (\text{total number of fruit})]$$

Triplicate analyses were performed for each treatment, with 10 fruits per replication.

2.4. Determination of Ethylene Production Rate

Ten pear fruits were sealed in a 9.5 L glass desiccator for 3 h at 25 °C. A 1.0 mL gas sample was withdrawn and injected into a gas chromatograph (GC97902 II; Fuli Chromatograph Instruments Co., Wenling, China) equipped with a GDX-102 column and a flame ionization detector. The column temperature was 78 °C, the injection temperature 120 °C, and the carrier gas N_2 at a flow rate of 40 mL min^{-1}. The ethylene production rate was expressed as ng kg^{-1} s^{-1}. Triplicate analyses were performed for each treatment.

2.5. Determination of CGA Content

CGA extraction and determination were carried out as described by He et al. [35]. Two grams of ground frozen core tissue was mixed with 10 mL of 80% (*v*/*v*) methanol under an ultrasonic oscillator for 20 min. After centrifugation at 10,000× *g* and 4 °C for 10 min, the supernatant was collected and subjected to solid phase extraction using a C18 column, washed with methanol and filtered through a 0.45 µm microporous membrane. The filtrate was collected for further analysis. CGA content was measured by high-performance liquid chromatography (HPLC) (HITACHI L2000 HPLC system), and expressed as g kg^{-1} fresh weight (FW). Each treatment included three replicates, and each replicate used 5 fruits.

2.6. Determination of Polyphenol Oxidase (PPO) Activity and Malondialdehyde (MDA) Content

For PPO extraction and activity analysis, 2.5 g of frozen core sample was homogenized in 10 mL of 100 mmol L^{-1} phosphate buffer (pH 7.8) containing 10% (*w*/*v*) polyvinyl pyrrolidone (PVP). After centrifugation at 20,000× *g* rpm and 4 °C for 15 min, the supernatant was collected as a crude PPO extract. PPO activity assay was initiated by the addition of 100 µL of PPO extract to the mixture of 3.0 mL of phosphate buffer (0.05 mol, pH 6.0) and 1.0 mL catechol (0.1 mol L^{-1}). The increase in absorbance at 420 nm was measured [32]. One unit of PPO activity was defined as an absorbance change of 0.1 at 420 nm per second, and expressed as U kg^{-1} FW.

MDA content was determined as described by Yuan et al. [44]. After homogenizing frozen core samples with 10% (*w*/*v*) trichloroacetic acid (TCA), the homogenate was centrifuged at 12,000× *g* rpm at 4 °C for 20 min. A 1.0 mL aliquot of the supernatant was mixed with 4.0 mL of 0.67% (*w*/*v*) thiobarbituric acid (TBA) and then the mixture was heated at 95 °C for 20 min, then placed in the ice-water bath for 5 min and centrifuged at 10,000× *g* rpm and 4 °C for 10 min. The supernatant was measured at 532 nm and 600 nm. MDA content was calculated according to its extinction coefficient (155 mmol L^{-1} cm^{-1}). Data are expressed as mmol kg^{-1} FW. Each treatment included three replicates, and each replicate used 5 fruits.

2.7. RNA Extraction and Quantitative RT-PCR (qRT-PCR) Analysis

Total RNA was isolated from the frozen core samples by the improved CTAB method [45]. After electrophoretic analysis, the first strand of cDNA was generated by reverse transcription using the PrimeScript RT reagent kit with gDNA eraser (Perfect Real Time) (Takara Bio Inc., Dalian, China).

QRT-PCR analysis was performed on an ABI 7500 Real-Time System (Applied Biosystems, Foster City, CA, USA) by using the SYBR Premix Ex TaqTM kit (Takara Bio Inc., Dalian, China).

The relative gene expression amount was calculated with the formula $2^{-\Delta\Delta CT}$ and *PbActin 2* was used as the internal reference [32] and the level of gene expression in the control at the initial time (day 0) was defined as 1. Primers of *PbACS3a*, *PbACS7* and *PbACS12* were consulted from Yuan et al. [46], and other primers were designed according to pear (*Pyrus bretschneideri*) nucleotide sequences registered in the GenBank of NCBI. The sequences of primers are shown in Supplementary Table S1. Each treatment included three replicates.

2.8. Statistical Analysis

The results were subjected to analysis of variance (ANOVA) and spearman's rank-order correlation by SPSS 18 software (SPSS Inc., Chicago, IL, USA). The least significant differences (LSD) were analyzed based on a level of 0.05. All values were expressed as means ± standard deviation (SD).

3. Results

3.1. Effects of AVG on the Fruit Quality and Core Browning

The firmness did not differ between the control and AVG-treated fruit at harvest, but it decreased with the extension of storage time. After 15 d of storage, the AVG-treated fruit maintained higher firmness than control (Figure 1A), and also, the AVG-treated fruit had a higher SSC at the end of storage (20 d and 25 d) (Figure 1B). There was no obvious difference in TA content between the AVG treatment and control at harvest, but a higher TA content was maintained in the AVG treatment than that in the control at the end of storage (Figure 1C).

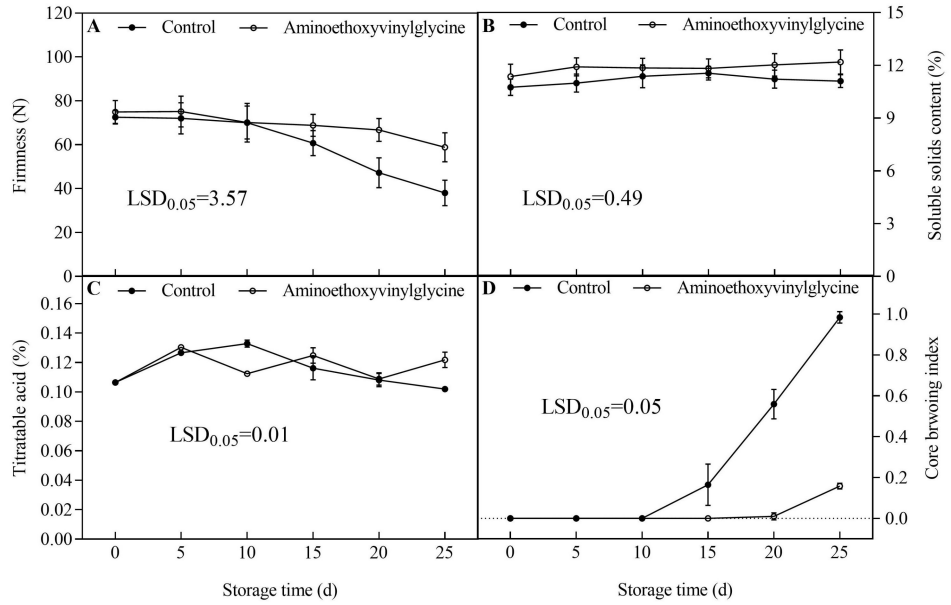

Figure 1. Effects of aminoethoxyvinylglycine (AVG) at 200 mg L^{-1} on fruit firmness (**A**), soluble solids content (SSC) (**B**), titratable acid (TA) content (**C**) and the core browning index (**D**) of 'Huangguan' pears during storage at 25 ± 1 °C. Values are means ± SD (n = 3).

Core browning appeared on day 15 of storage and afterwards was much more obvious in the control fruit. AVG treatment significantly reduced core browning and delayed its appearance by 10 days (Figure 1D).

3.2. Effect of AVG on Ethylene Production Rate

The ethylene production rate of fruit increased gradually at first, and reached its maximum level at day 20 in the control fruit, but it was at a significantly lower level with a slowly increasing trend in AVG-treated fruit (Figure 2).

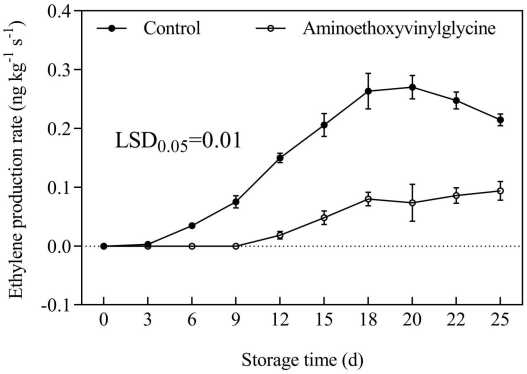

Figure 2. Effect of aminoethoxyvinylglycine (AVG) at 200 mg L^{-1} on the ethylene production rate of 'Huangguan' pears during storage at 25 ± 1 °C. Values are means ± SD (n = 3).

3.3. Effect of AVG on CGA and MDA Contents and PPO Activity in the Core Tissue

The CGA content increased slowly at first, reached maximum value at day 15 and 20 in the control and AVG-treated fruit, respectively, and then decreased (Figure 3A). This indicated that AVG treatment could delay the high accumulation of CGA by 5 days. The MDA content increased in both the AVG treatment and control during storage, and it was significantly lower in the AVG treatment than in the control (Figure 3B). The PPO activity increased markedly after day 20 of storage in the control, but it increased slowly and remained at a lower level in the AVG treatment (Figure 3C).

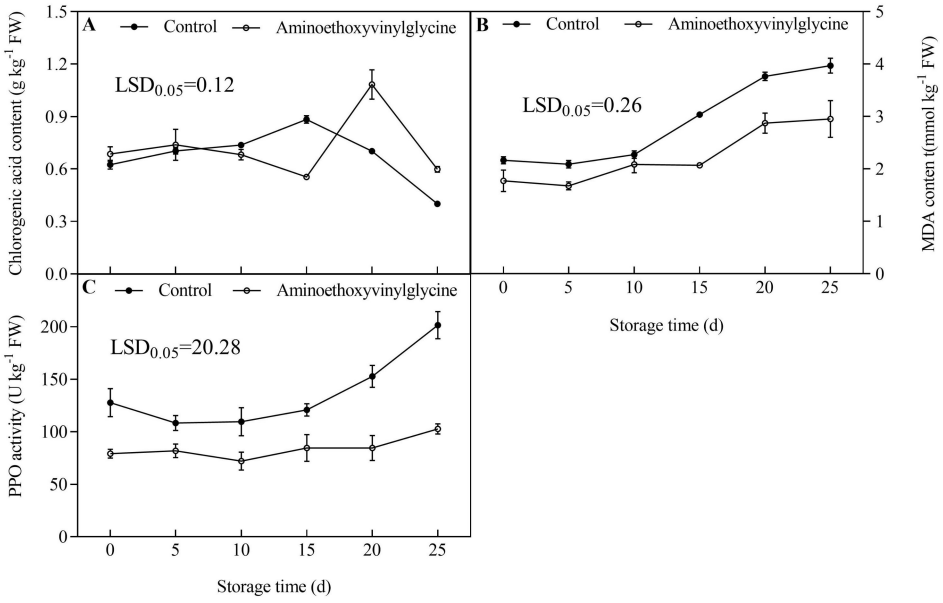

Figure 3. Effects of aminoethoxyvinylglycine (AVG) at 200 mg L^{-1} on chlorogenic acid (CGA) (**A**), malondialdehyde (MDA) (**B**) contents and polyphenol oxidase (PPO) activity (**C**) of core tissue in 'Huangguan' pears during storage at 25 ± 1 °C. Values are means ± SD (n = 3).

3.4. Effect of AVG on the Expression of Genes Associated with Ethylene Biosynthesis and Signal Transduction in Core Tissue

The present results showed that the expression levels of *PbACO1*, *PbACO2*, *PbACS2*, *PbACS3a*, *PbACS5a* and *PbACS5b* increased gradually and afterwards reached maximum values in the control, which were significantly reduced by AVG treatment (Figure 4A–F). However, the changes of *PbACS10a* and *PbACS12* expression showed no obvious variation rules in two treatments, and the *PbACS7* did a different expression pattern from other *ACS* genes, having a much higher expression level in the AVG treatment than control at late stage (Figure 4G–I).

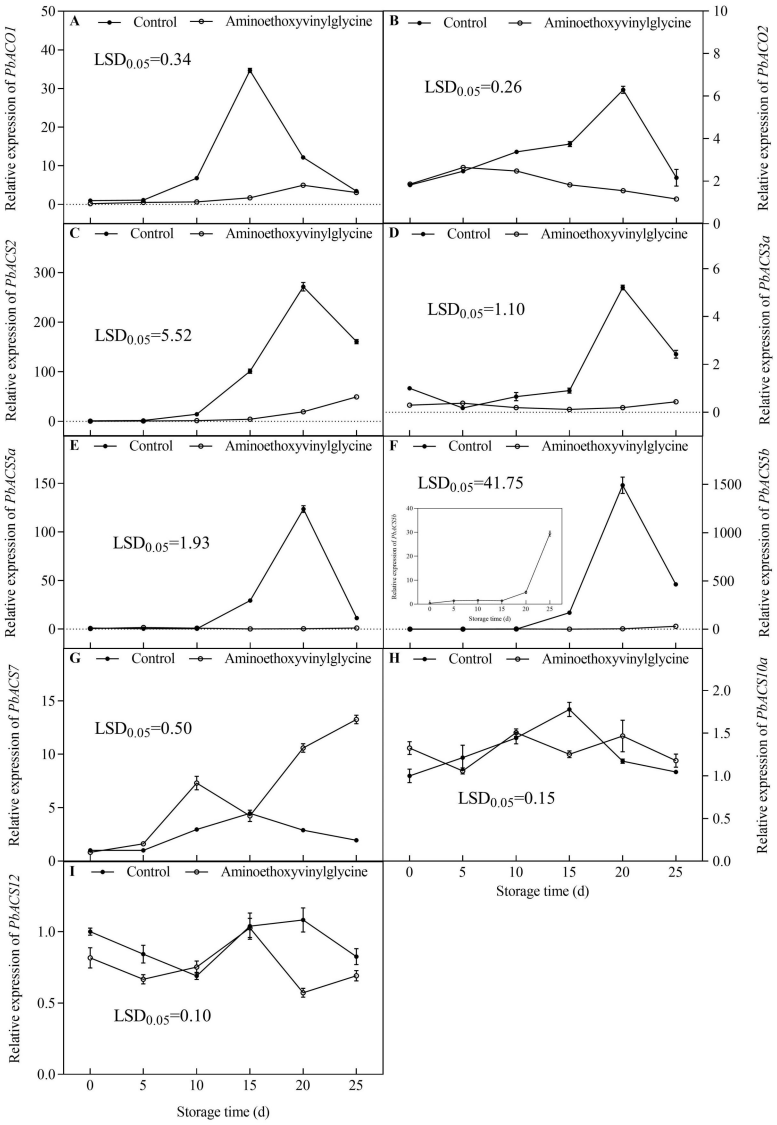

Figure 4. Effects of aminoethoxyvinylglycine (AVG) at 200 mg L^{-1} on the expression of genes associated with ethylene biosynthesis ((**A**): *PbACO1*, (**B**): *PbACO2*, (**C**): *PbACS2*, (**D**): *PbACS3a*, (**E**): *PbACS5a*, (**F**): *PbACS5b*, (**G**): *PbASC7*, (**H**): *PbACS10a*, (**I**): *PbACS12*) in the core tissue of 'Huangguan' pears during storage at 25 ± 1 °C. Values are means ± SD (n = 3).

The expression of *PbETR2* and *PbERS1* increased obviously during early storage, and then decreased after reaching its peak value at 15 d (control) and 20 d (AVG) (Figure 5A). *PbERS1* increased first; the control fruit maintained maximum expression on day 15 and 20. The expression of *PbERS1* increased and reached a higher level on day 10, then was maintained with minimal changes (Figure 5A,B). The expression levels of *PbETR2* and *PbERS1* were markedly down-regulated by AVG to varying degrees. The expression level of *PbERF1* was very low in the early period of storage, but increased rapidly after day 20 in the control, whereas it was much lower in AVG treatment throughout the entire storage period (Figure 5C).

3.5. Effect of AVG on the Expression of Genes Associated with CGA Biosynthesis in the Core Tissue

The expression of *PbPAL1* seldom changed within 10 d of storage, and then increased significantly until day 15 in the control, but it barely changed and was markedly lower in AVG-treated fruit (Figure 6A). The expression of *Pb4CL2* and *PbHCT1* increased gradually during storage in the control, but it was significantly inhibited by AVG after 15 d of storage

(Figure 6B,C). The expression of *PbHCT3* increased significantly after day 20 in the control, but it was significantly decreased by AVG treatment (Figure 6D). The expression of *PbC4H4* had a similar pattern to that of *PbPAL1*, and it sharply increased after 20 days of storage in the control and was significantly inhibited by AVG treatment (Figure 6E).

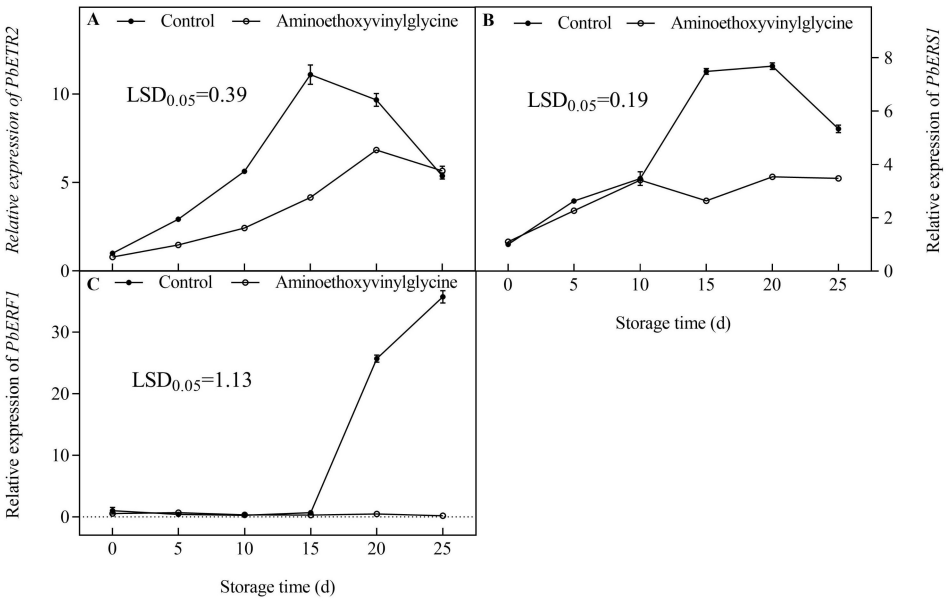

Figure 5. Effects of aminoethoxyvinylglycine (AVG) at 200 mg L^{-1} on the expression of genes associated with ethylene receptors ((**A**): *PbETR2*, (**B**): *PbERS1*)and response factors ((**C**): *PbERF1*) in the core tissue of 'Huangguan' pears during storage at 25 ± 1 °C. Values are means ± SD (n = 3).

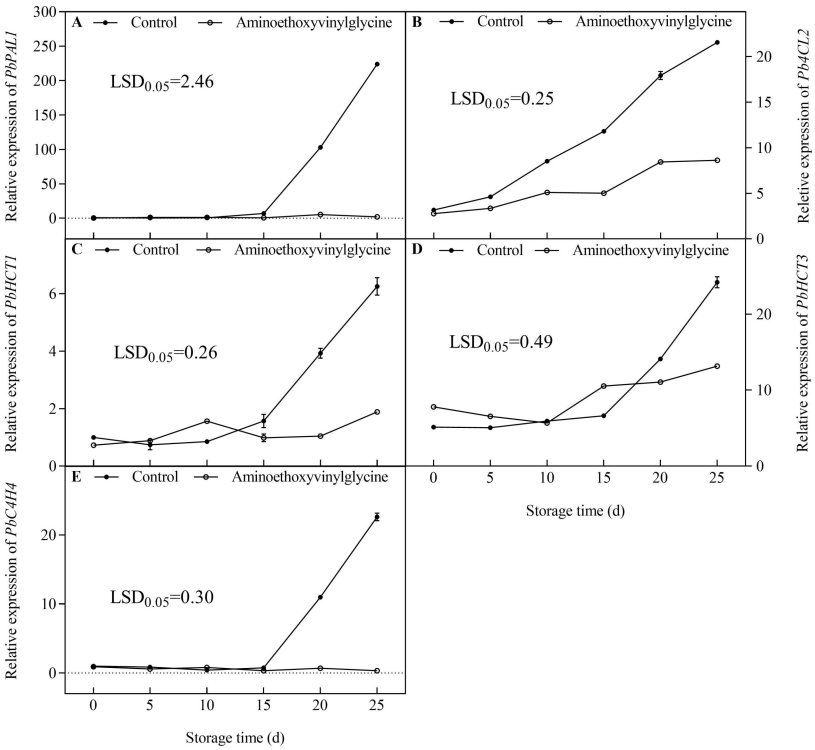

Figure 6. Effects of aminoethoxyvinylglycine (AVG) at 200 mg L^{-1} on the expression of genes associated with phenolic biosynthesis ((**A**): *PbPAL1*, (**B**): *Pb4CL*, (**C**): *PbHCT1*, (**D**): *PbHCT3*, (**E**): *PbC4H4*) in the core tissue of 'Huangguan' pears during storage at 25 ± 1 °C. Values are means ± SD (n = 3).

3.6. Effect of AVG on the Expression of the PbPPO1 and PbPPO5 Genes in the Core Tissue

The expression of *PbPPO1* increased significantly after 20 days of storage in the control fruit, but it was significantly reduced by AVG treatment (Figure 7A). The expression of *PbPPO5* was sharply up-regulated after 15 d of storage in the control, but it was significantly inhibited by AVG treatment (Figure 7B). According to the transcript amount, the increased extent of *PbPPO5* expression was much higher than that of *PbPPO1* (Figure 7A,B).

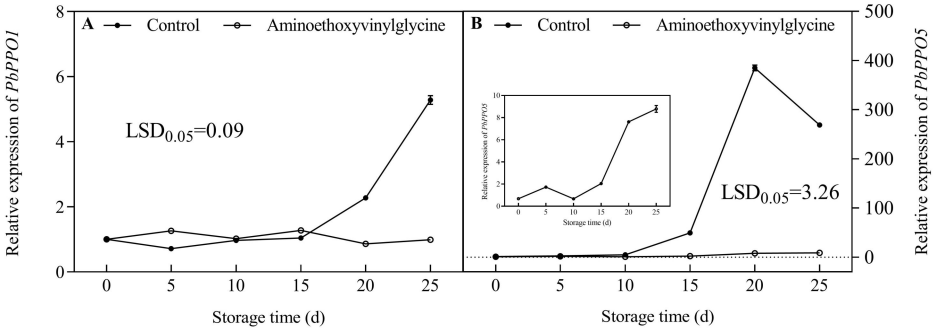

Figure 7. Effects of aminoethoxyvinylglycine (AVG) at 200 mg L^{-1} on the expression of *PbPPO1* (**A**) and *PbPPO5* (**B**) in the core tissue of 'Huangguan' pears during storage at 25 ± 1 °C. Values are means ± SD (n = 3).

3.7. Effect of AVG on the Expression of PbLOX1, PbLOX5 and PbPLD1 in the Core Tissue

The expression level of *PbLOX1* increased gradually during storage, and it was significantly lowered by AVG treatment (Figure 8A). The expression of *PbLOX5* gradually increased before day 15, reached its peak on day 20, and then declined sharply in the control; however, it increased slightly and remained at a much lower level in the AVG treatment (Figure 8B). The expression of *PbPLD* increased significantly from day 5 to day 15, and then decreased in the control; in contrast, it was lower and its peak was delayed by 5 days in the AVG treatment (Figure 8C).

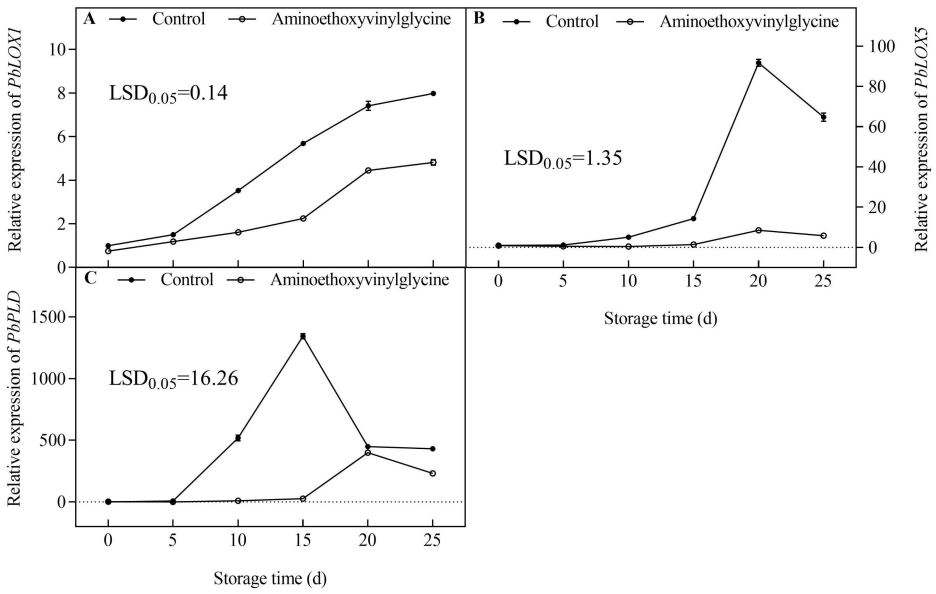

Figure 8. Effects of aminoethoxyvinylglycine (AVG) at 200 mg L^{-1} on the expression of *PbLOX1* (**A**), *PbLOX5* (**B**) and *PbPLD* (**C**) in the core tissue of 'Huangguan' pears during storage at 25 ± 1 °C. Values are means ± SD (n = 3).

3.8. Correlation Analysis of Core Browning Index with Gene Expression Levels

Correlation analysis showed that there was a significant and positive correlation between the core browning index and the expression of CGA metabolism-related genes (*PbPAL1*, *Pb4CL2*, *PbHCT1*, *PbHCT3* and *PbPPO5*), ethylene biosynthesis and signaling pathway-associated genes (*PbACO1*, *PbACS2*, *PbACS3a*, *PbACS5a*, *PbACS5b*, *PbETR2* and *PbERS1*), and the genes involved in cell membrane breakdown (*PbLOX1*, *PbLOX5* and *PbPLD*) (Figure 9). This indicated that the expression patterns of the above-mentioned genes were synchronous with the process of core browning.

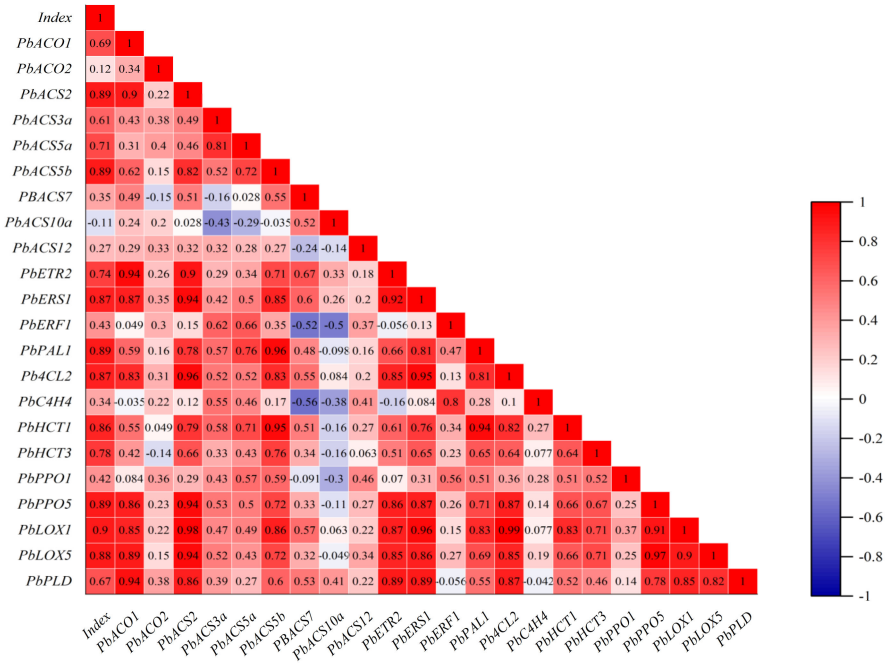

Figure 9. Correlation analysis of the core browning index with the transcript amounts of detected genes in the core tissue of 'Huangguan' pears (n = 12, $r_{0.05}$ = 0.576, $r_{0.01}$ = 0.708).

4. Discussion

AVG treatment significantly reduced the ethylene production of 'Huangguan' pear and delayed its peak value during storage (Figure 2), and maintained higher fruit firmness (Figure 1A) and SSC (Figure 1B), significantly reducing the core browning index (Figure 1D). In nectarine, AVG treatment significantly delayed fruit softening along with a lower ethylene production, but prevented the increase in SSC [8]. In the study of 'McIntosh' apple, AVG-treated fruit exhibited a higher firmness but lower SSC accompanied by lower internal ethylene concentration [9]. In pears, AVG treatment suppressed ethylene production, respiration rate and reductions in fruit firmness, and reduced senescence disorders [15,16]. In this study, the effective function of AVG on the suppression of ethylene production was confirmed, resulting in slower fruit softening and less core browning. However, higher SSC was found in the AVG-treated fruit at day 20 and 25. Thus, these results indicated that AVG treatment could delay fruit ripening and maintain higher fruit quality during storage.

AVG reduces ethylene production by reducing ACS activity [2], and by down-regulating ACS-associated gene expression [11,16,47]. In peach fruit, AVG treatment delayed the *PpACO1* expression peak, but caused an increase in *PpETR1* and *PpERS1* transcript [11]. In 'Starkrimson' pear, AVG suppressed the expression of ethylene synthesis (*PcACS1*, *PcACS4*, *PcACS5* and *PcACO1*) and perception genes (*PcETR1*, *PcETR2* and *PcETR5*) [16]. In Golden Delicious apple, AVG efficiently reduced the ethylene evolution, and down-regulated the expression of *MdACS1*, *MdACO1*, *MdETR1* and *MdERS1* [47]. In this work, AVG treatment significantly repressed the expression level of *PbACO1*, *PbACO2*, *PbACS2*, *PbACS3a*, *PbACS5a* and *PbACS5b* (Figure 4A–F), along with a reduction in the ethylene production

(Figure 2). Further investigation showed that AVG treatment significantly inhibited the gene expression of ethylene receptors (*PbETR2* and *PbERS1*) and ethylene response factor (*PbERF1*) (Figure 5).

CGA is involved in the enzymatic browning catalyzed by PPO [30,31,34,35]. In the present study, AVG differently down-regulated the expression of genes associated with CGA biosynthesis during storage (Figure 6), and delayed the peak of CGA by 5 days (Figure 3A). *PAL1* is an important gene involved in CGA biosynthesis [22] and core browning [29] in pears. The increase in PPO activity and upregulated expression of the *PbPPO*s was closely related to core browning in pear [32,33,43]. In this study, AVG significantly decreased the PPO activity (Figure 3C) and the transcription levels of *PbPPO1* and *PbPPO5* (Figure 7). Especially, the sharply up-regulated *PbPPO5* expression was synchronous with the occurrence of core browning in the control (Figures 1D and 7B), whereas it was maintained at an extremely low level in AVG treatment (Figure 7B), suggesting that *PbPPO5* might play a relatively important role in the initiation of core browning. Interestingly, the higher CGA content was found after day 20 in the AVG treatment (Figure 3A). In addition, the correlation analysis (Supplementary Table S2) showed that the core browning index was significantly positive correlated with the activity of PPO and content of MDA, but not significantly with the content of CGA. This result might be related to the overall effect of the biosynthesis and oxidant processes of CGA, and further suggests that the role of PPO was more important than that of CGA in the process of core browning.

LOX and PLD are two important enzymes associated with cell membrane breakdown, generating hydroperoxides from free di- and tri-enoic fatty acids and resulting in the production of reactive oxygen species (ROS). ROS initiates the lipid catabolism, senescence and browning processes [36–41,48–50]. In this study, AVG treatment down-regulated the expression of *PbLOX1*, *PbLOX5* and *PbPLD* (Figure 8), suggesting that AVG treatment can avoid the loss of membrane function, and potentially protect membrane lipids from oxidative damage. This finding is consistent with the lower MDA content in the AVG-treated fruit (Figure 3B). MDA, as a marker of cell membrane lipid peroxidation, is involved in the fruit browning [51,52]. Our results suggested that AVG reduced membrane lipid peroxidation, and favored cell membrane integrity and cellular compartmentalization and subsequently mitigated the release of phenolic compounds from the vacuole and PPO from the plastids, thereby repressing the PPO-catalyzed phenolic oxidation to o-quinones and reducing core browning.

Correlation analysis showed that the core browning index was significantly and positively correlated with the expression of *PbACO1*, *PbACS2*, *PbACS3a*, *PbACS5a*, *PbACS5b*, *PbETR2*, *PbERS1*, *PbPAL1*, *Pb4CL2*, *PbHCT1*, *PbHCT3*, *PbPPO5*, *PbLOX1*, *PbLOX5* and *PbPLD* (Figure 9). This suggested that these gene transcripts are closely related to the development of core browning, and additionally, the regulation of the ethylene biosynthesis and signaling pathway, which are closely associated with CGA metabolism and cell membrane breakdown. However, there was no significant linear correlation between the core browning index and the expression amount of *PbACO2* and *PbERF1*, even though there was an increase in the expression of *PbACO2* and *PbERF1* prior to the onset of core browning (Figures 1D, 4B and 5C), indicating that these two genes might be involved in the initiation of core browning. This is a subject deserving of further study.

5. Conclusions

AVG at 200 mg L^{-1} decreased the 'Huangguan' fruit ethylene production rate and significantly inhibited the expression of genes associated with CGA metabolism, ethylene biosynthesis, perception and signal transduction and membrane breakdown in core tissue, as well as decreasing the PPO activity and MDA content. Thus, AVG effectively delayed fruit ripening and reduced core browning in 'Huangguan' pears.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020179/s1, Figure S1: Core browning index af-

fected by AVG treatment with different concentrations; Table S1: Sequence of primers for qRT-PCR; Table S2: Correlation analysis of the core browning index with the content of CGA and MDA, activity of PPO in the core tissue of 'Huangguan' pears.

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