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[1]Institute of Genetics and Physiology, Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang, China
[2]Plant Genetic Engineering Center of Hebei Province, Shijiazhuang, China

Effects of 1-methylcyclopropene on superficial scald and related metabolism in 'Wujiuxiang' pear during cold storage

Manman Gao[1, 2 a], Shuo Zhou[1, 2 a], Junfeng Guan[1, 2 *], Yingying Zhang[1, 2]

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Summary

'Wujiuxiang' (*Pyrus bretschneideri* R. × *Pyrus communis* L.) pear often suffers from superficial scald after a long-term of cold storage. In this study, harvested 'Wujiuxiang' pear fruits were fumigated with 1-methylcyclopropene (1-MCP) at concentrations of 0.5 µL/L and 1.0 µL/L and subsequently stored at low temperature (0 °C). The superficial scald index; flesh firmness; total soluble solids (TSS) content; respiration and ethylene production rates; relative membrane permeability; contents of α-farnesene, conjugated trienols (CTols) and hydrogen peroxide (H_2O_2); and activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and lipoxygenase (LOX) of the peel were investigated. The results showed that compared with control, 1-MCP reduced the index of superficial scald; maintained a higher firmness and a lower TSS content; inhibited the accumulation of H_2O_2, α-farnesene and conjugated trienols and the increase in cell membrane permeability; and maintained a higher activity of APX, SOD and CAT and a lower activity of LOX. These findings indicate that 1-MCP regulates the activities of H_2O_2-scavenging enzymes to inhibit the accumulation of H_2O_2 and thereby reduces cell membrane damage and inhibits the accumulation of conjugated trienols. Thus, 1-MCP could decrease the incidence of superficial scald in 'Wujiuxiang' pear.

Abbreviations
1-MCP, 1-methylcyclopropene; **TSS**, total soluble solids; **CTols**, conjugated trienols; **H_2O_2**, hydrogen peroxide; **SOD**, superoxide dismutase; **CAT**, catalase; **APX**, ascorbate peroxidase; **LOX**, lipoxygenase

Introduction

'Wujiuxiang' (*Pyrus bretschneideri* R. × *Pyrus communis* L.) pear is favored for their excellent quality of flavor and exhibit a high market value. However, this fruit is highly susceptible to superficial scald, which is a physiological disorder that manifests as many irregular brown or black patches on the fruit skin after more than 90 days of cold storage and during subsequent shelf life (Dong et al., 2012), resulting in reduced fruit quality and economic loss.

It has been suggested that scald development in apple and pear is related with the accumulation of α-farnesene and its oxidation products, namely, conjugated trienols (CTols), which accumulate progressively in the fruit peel during storage (Du and Bramlage, 1993; Giné Bordonaba et al., 2013; Whitaker, 2007). Some studies have also shown that antioxidative enzyme activity and H_2O_2 accumulation are correlated with superficial scald development (Abbasi et al., 2008; Ahn et al., 2007; Larrigaudiere et al., 2004; Sabban-Amin et al., 2011). H_2O_2 accumulation may cause lipid peroxidation, which may result in cell senescence and superficial scald (Lu et al., 2011; Rao et al., 1998; Sabban-Amin et al., 2011; Tian et al., 2013). Many postharvest methods have been established to control scald development; however, the mechanism of superficial scald development has not yet been completely understood (Lurie and Watkins, 2012).

1-Methylcyclopropene (1-MCP), an ethylene action inhibitor, can effectively extend the shelf life and maintain a higher quality of many types of fruits, and it has been shown that 1-MCP can lower ethylene production, delay softening, prevent chlorophyll degradation, retard the decline in protein, reduce the respiration rate and volatiles and alter the titratable acidity and sugar contents (Blankenship and Dole, 2003; Watkins, 2006). Previous work has shown that 1-MCP has significant effects on inhibiting superficial scald development in 'Granny Smith' apple (Zanella, 2003), 'Cortland' and 'Delicious' apples (Lu et al., 2013), 'Rocha' pear (Isidoro and Almeida, 2006), 'Anjou' pear (Argenta et al., 2003; Bai et al., 2009), 'Dangshansuli' pear (Hui et al., 2011), 'Akemizu' pear (Li and Wang, 2009), and 'Asian' pear (Yazdani et al., 2011). Therefore, the application of 1-MCP for the control of scald development has become a promising technology and research direction.

It has been shown that 1-MCP can inhibit scald development by delaying the production of α-farnesene and conjugated trienols in pear and apple fruits (Ekman et al., 2004; Isidoro and Almeida, 2006; Lu et al., 2013; Yazdani et al., 2011). 1-MCP can also retain higher activities of antioxidative enzymes and inhibit H_2O_2 accumulation and lipid peroxidation in fruit, which may reduce scald incidence during storage (Chiriboga et al., 2013a; Dong et al., 2011; Larrigaudiere et al., 2004; Li and Wang, 2009). However, research on the regulation mechanism of 1-MCP in scald development at cold storage is still relatively lacking.

Therefore, the objectives of this study were to investigate (1) the effect of 1-MCP on superficial scald development and (2) the possible mechanism of 1-MCP in inhibiting scald development in 'Wujiuxiang' pear during cold storage.

Materials and methods

Materials and treatments

'Wujiuxiang' pear fruits were harvested in Jinzhou County (Hebei, China) at the fruit commercial mature stage (August 29, 2010). The harvested fruits were transported to the laboratory within 2 h. Approximately 750 kg fruits that exhibited uniformity of weight (average weight per fruit 289.26 g), shape and color without any visual defects were selected to take three treatments: untreated control, 0.5 and 1.0 µL/L 1-MCP fumigation. 1-MCP (Rohm and Haas China Inc., Beijing) fumigation treatments at 0.5 or 1.0 µL/L were carried out using sealed plastic containers for 24 h at 25 ± 2 °C. Untreated control was sealed with air. After treatment, each group was subsequently stored at 0 °C. All experiments in this paper were conducted directly from cold storage without shelf life. Peel samples were periodically collected, quickly frozen in liquid nitrogen and stored in a freezer at -80 °C.

Methods

Scald index

The scald index values were monitored throughout the cold storage. The scald data are expressed as a scald index based on the percentage

* Corresponding author
[a] These authors contributed equally to the article.

of the fruit surface area affected (Zanella, 2003), where no scald = 0, < 25% = 1, 25-50% = 2, and > 50% = 3. The scald index was normalized to 100 by multiplying the values by 100/3. Three replicates were measured for each treatment, and 10 fruits were included in each replicate.

Firmness and TSS content

The flesh firmness was measured on two opposite points on the equatorial line of each fruit after peel removal using a fruit hardness tester (TuoPu Instruments, Zhejiang, China; Model GY-J) mounted on a standard drill press and fitted with an 8-mm probe.

The TSS content (°Brix) was determined with a digital refractometer (Atago, Japan; Model PAL-1) by measuring the refractive index of the juice extracted from the same fruits used to determine firmness. Two opposite points per fruit were selected to determine the firmness and TSS content of each treatment, and three replicates were measured for each treatment with 10 fruits per replicate.

Respiration rate

The respiration rate was measured using an infrared carbon dioxide analyzer (Kexi Instruments, Jiangsu, China; Model HWF-1A) at 0 °C after the fruits were sealed in glass desiccators (9.35 L) for 30 min. Then, a 1-mL sample of gas was withdrawn with a syringe to analyze the respiration rate. The result was expressed as mg CO_2 kg^{-1} FW h^{-1}. Three replicates were measured for each treatment with 10 fruits per replicate.

Ethylene production rate

The pear fruits were randomly selected and sealed in glass desiccators (9.35 L) for 4 h at 0 °C. A 1-mL sample of the headspace gas was withdrawn with a gas-tight syringe from each desiccator through a septum stopper and injected into a gas chromatograph (Kechuang Instruments, Shanghai, China; Model GC-9800) that was equipped with a GDX-502 column and a flame ionization detector (FID). The column temperature was 78 °C, and the injection temperature was 120 °C. The carrier gas was N_2 with a rate of 40 mL min^{-1}, and the rate of ethylene production was expressed as μL kg^{-1} FW h^{-1}. Three replicates were measured for each treatment with 10 fruits per replicate.

α-farnesene and conjugated trienols content

The extraction and analysis of α-farnesene and conjugated trienols were performed as described by Anet et al. (1972) and Isidoro and Almeida (2006) with some modifications. Four discs (1 cm in diameter) were removed from one strip of peel taken from the equatorial portion of each pear. These discs were placed in tubes containing 20 mL of hexane for 2 h. Two milliliters of the extract was filtered through a Florisil column and eluted using 3 mL of hexane for measurement of the absorbance at 232 nm. The absorbance of the remaining extract was measured at 281 and 290 nm. The contents of *a*-farnesene and conjugated trienols per cm^2 of pear peel were calculated using the molar extinction coefficients $\varepsilon_{232} = 27{,}740$ for α-farnesene and $\varepsilon_{281-290} = 25{,}000$ for the conjugated trienols. Three replicates were analyzed for each treatment with 10 fruits per replicate.

H_2O_2 content

The H_2O_2 content was measured according to the method described by Bellincampi et al. (2000) with some modifications. Four grams of fresh peel were homogenized in 8 mL of 100 mM sodium phosphate buffer (pH 7.2) with 10% (w/v) polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 10,000 g for 10 min at 4 °C. Then, 500 μL of the supernatant fractions was added to 1 mL of assay reagent (500 μM ammonium ferrous sulfate, 50 mM H_2SO_4, 200 mM xylenol orange, and 200 mM sorbitol) and 500 μL of 100 mM sodium phosphate buffer (pH 7.2). The absorbance at 560 nm was detected after 30 min of dark incubation. Standard curves of H_2O_2 were obtained for each independent experiment by adding variable amounts of H_2O_2 to 1 mL of basal medium mixed with 1 mL of assay reagent. The data were normalized and expressed as nmol g^{-1} FW. Three replicates were measured for each treatment with 10 fruits per replicate.

Relative electrolyte leakage rate

The relative electrolyte leakage was used as an indicator of the cell membrane permeability and was measured using a conductivity meter (YiDian Scientific Instruments, Shanghai, China; Model LeiCi DDS-307) as described by Wang et al. (2005) with some modifications. Four discs (1 cm in diameter) were removed from one strip of peel taken from the equatorial portion of each pear. These discs were placed in tubes containing 20 mL of distilled H_2O for 1 h at ambient temperature (25 °C), and the initial conductivity was then measured. The samples were boiled for 30 min to release all electrolytes, and after the temperature dropped to ambient temperature, the final conductivity was measured. The percentage of electrolyte leakage was calculated as the ratio (×100) of the conductivity measurements before and after boiling. Three replicates were measured for each treatment, and 10 fruits were included in each replicate.

Enzymes extractions and activities

SOD and CAT

Two grams of frozen peel were ground in liquid N_2 in a mortar and pestle. The completely grinding powder was blended in 5 mL of 100 mM phosphate buffer (pH 7.8), 2 mM DTT, 5% (w/v) polyvinylpolypyrrolidone (PVPP), 0.1 mM EDTA and 1.25 mM polyethylene glycol. The extract was centrifuged at 12,000×g for 30 min at 4 °C. The supernatants were transferred to fresh tubes as the crude enzyme extract for the measurements of SOD and CAT (Larrigaudière et al., 2004; Zavaleta-Mancera et al., 2007).

The SOD (EC 1.15.1.1) activity was assayed according to Larrigaudiere et al. (2004) with some modifications and determined by measuring the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture was composed of 1.5 mL of 50 mM phosphate buffer (pH 7.0), 0.3 mL of 130 μM L-methionine, 0.3 mL of 750 μM NBT, 0.3 mL of 100 μM EDTA-Na_2, 0.3 mL of 20 μM riboflavin, 200 μL of H_2O, and 100 μL of the crude enzyme extract. The reaction was initiated by illuminating the assay mixture with 60 mmol m^{-2} s^{-1} white fluorescent light at 25 °C. After 8 min, the reaction was stopped by removing the light source. A control tube with the assay mixture and enzyme was maintained in the dark, while another tube without the enzyme was maintained in light conditions to serve as the control for the reduction of NBT by light. The absorbance of the reaction was read at 560 nm. The activity of SOD is reported as the NBT reduction in light without enzyme minus the NBT reduction with enzyme. One unit of SOD activity is defined as the amount of enzyme that inhibits the NBT photoreduction by 50% under the assay conditions. The SOD data are presented as units of activity per mg protein.

The CAT (EC 1.11.1.6) activity was assayed according to Zavaleta-Mancera et al. (2007) with some modifications. The assay mixture (3 mL) contained 50 mM phosphate buffer (pH 7.0) and 100 μL of the crude enzyme extract. The reaction was initiated by the addition of 100 μL of 0.1 M H_2O_2. The decomposition was followed directly

by the decrease in absorbance at 240 nm every 30 s for 2 min at 25 °C. One unit of CAT activity is defined as the amount of enzyme that reduces the absorbance by 0.01 in one minute.

APX

The extraction of the APX enzyme was performed using 100 mM phosphate buffer (pH 7.0), 2 mM DTT, and 5 mM ascorbate (Zavaleta-Mancera et al., 2007). The APX (EC 1.11.1.11) activity was determined by monitoring the decomposition of H_2O_2 at 290 nm according to the method described by Zavaleta-Mancera et al. (2007) with some modifications. The reaction mixture (2 mL) contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM ascorbate, 2.7 mM H_2O_2 and 100 µL of the crude enzyme extract. The reaction was initiated by the addition of H_2O_2. The decrease in absorbance due to the oxidation of ascorbate was determined at 290 nm every 30 s for 3 minutes at 25 °C. One unit of APX activity is defined as the amount of enzyme that oxidizes 1 µM ascorbate in one minute.

LOX

The LOX enzyme was extracted using 100 mM phosphate buffer (pH 7.5), 2 mM DTT, 1 mM EDTA, 0.1% (v/v) Triton X-100, and 1% PVPP (Axelrod et al., 1981). The LOX (EC 1.13.11.12) activity was assayed according to Axelrod et al. (1981) with some modifications using sodium linoleate as the substrate. The substrate was prepared as follow: 28 µL of sodium linoleate and 36 µL of Tween-20 were mixed in 8 mL of oxygen-free water, and 2 M NaOH was added to clear the solution. Finally, the volume of the solution was set to 10 mL with distilled water. The assay mixture (3 mL) contained 2.925 mL of 200 mM phosphate buffer (pH 7.0), 25 µL of the substrate solution and 50 µL of the crude enzyme extract. The reaction was initiated by the addition of the crude enzyme extract. The absorbance of the mixture was recorded at 234 nm every 1 min for 8 minutes at 25 °C. The LOX activity is expressed as ΔOD_{234} min^{-1} mg^{-1} protein.

The protein contents of above enzymes were determined according to the method described by Bradford (1976) using bovine serum albumin as the standard. Three replicates were extracted and measured for each treatment with 10 fruits per replicate.

Statistical analysis

All of the data were analyzed by one-way analysis of variance (ANOVA) according to treatment. All of the values are expressed as the means ± SE of three replicates. Differences were considered significant at $P < 0.05$. All of the analyses were performed with SPSS 12.0 software (SPSS Inc. Chicago, IL, USA).

Results

Effects of 1-MCP on superficial scald index and quality

During cold storage, superficial scald was observed after 120 days and was lowered by 1-MCP treatment, particularly by 1-MCP with the concentration of 1.0 µL/L (Fig. 1A). These findings suggested that the 1.0 µL/L concentration of 1-MCP was more effective for reducing the scald incidence.

The firmness declined in all of the treatments during the storage period, whereas the 1.0 µL/L 1-MCP-treated fruits showed higher firmness than the control ($P < 0.05$, Fig. 1B). On the contrary, the contents of TSS increased in all of the treatments, and a lower value was obtained in the 1.0 µL/L 1-MCP-treated fruits ($P < 0.05$, Fig. 1C). This result indicated that 1-MCP could delay fruit softening and senescence.

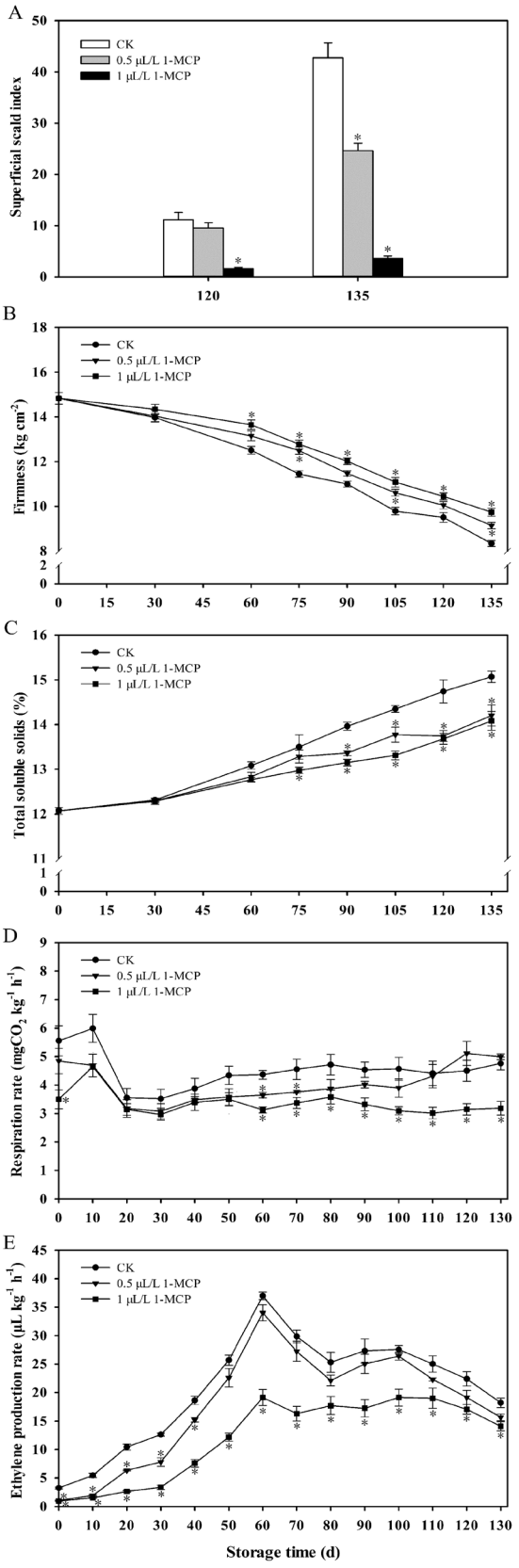

Fig. 1: Effects of 1-MCP on superficial scald index (A), firmness (B), total soluble solids (C), respiration rate (D), and ethylene production rate (E) in 'Wujiuxiang' pear fruits during storage at 0 °C. The data are the means ± SE of three replicates. The vertical bars represent the standard errors of the means. Star represents significant difference ($P < 0.05$).

Effects of 1-MCP on respiration and ethylene production rates

The respiration rates of 'Wujiuxiang' pear fruits were relatively stable during cold storage, and no peak was observed. However, the respiration rates of the 1-MCP-treated fruits were generally lower than those of the control after 60 days of storage, and this difference was particularly observed with the 1.0 µL/L 1-MCP treatment from control ($P < 0.05$, Fig. 1D).

The ethylene production rates increased steadily in the control and the 0.5 µL/L 1-MCP-treated fruits until 60 days and then declined, whereas it increased markedly slower in the 1.0 µL/L 1-MCP-treated fruit before 60 days and then remained at a lower level than that observed in the control ($P < 0.05$, Fig. 1E).

Effects of 1-MCP on contents of α-farnesene and conjugated trienols

In the control fruit, α-farnesene accumulated from an initial value of 6.58 nmol per cm^2 to a peak of 81.56 nmol per cm^2 after 60 days in storage and declined thereafter. The α-farnesene accumulation in the fruits treated with 1-MCP showed similar trends; however, it maintained a lower level with 1.0 µL/L 1-MCP than those with 0.5 µL/L 1-MCP and control (Fig. 2A).

The contents of conjugated trienols in all treated fruits increased at later storage period; however, it accumulated less in fruits with 1.0 µL/L 1-MCP than those with 0.5 µL/L 1-MCP and control, particularly before 105 days of storage (Fig. 2B).

Effects of 1-MCP on H_2O_2 accumulation and activities of antioxidant enzymes

H_2O_2 accumulated progressively in the peel during cold storage, especially after 60 days. Compared with the control, 1-MCP could effectively inhibit the accumulation of H_2O_2, especially in the treatment with 1.0 µL/L ($P < 0.05$, Fig. 3A).

The SOD activity in the peel steadily increased in all of the treatments until 90 days of storage and then declined. The 1-MCP-treated fruits maintained higher levels of SOD activity than the control, and the 1.0 µL/L 1-MCP-treated ones exhibited the highest levels (Fig. 3B). Similar results were also observed with the CAT and APX activities. However, the peaks of CAT and APX activities occurred on 105 d and were 15 days later than that of SOD activity (Fig. 3C and D).

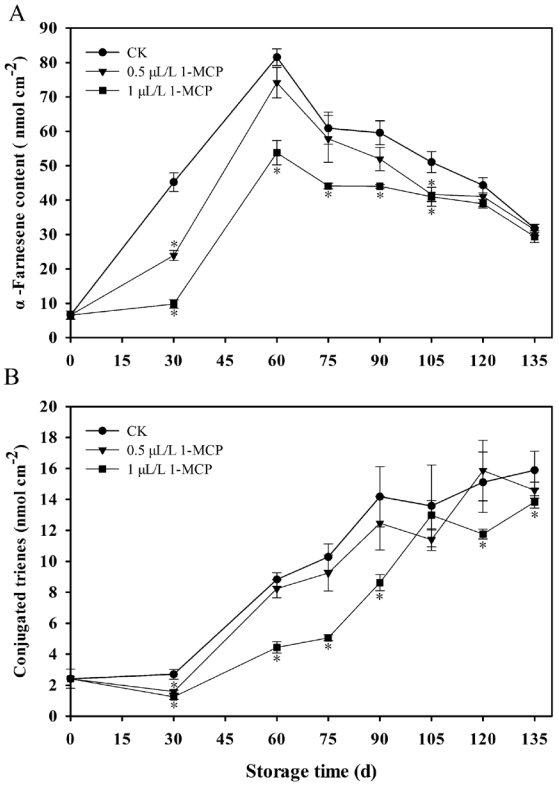

Fig. 2: Effects of 1-MCP on the accumulation of α-farnesene (A) and conjugated trienols (B) in 'Wujiuxiang' pear fruits during storage at 0 °C. The data are the means ± SE of three replicates. The vertical bars represent the standard errors of the means. Star represents significant difference ($P < 0.05$).

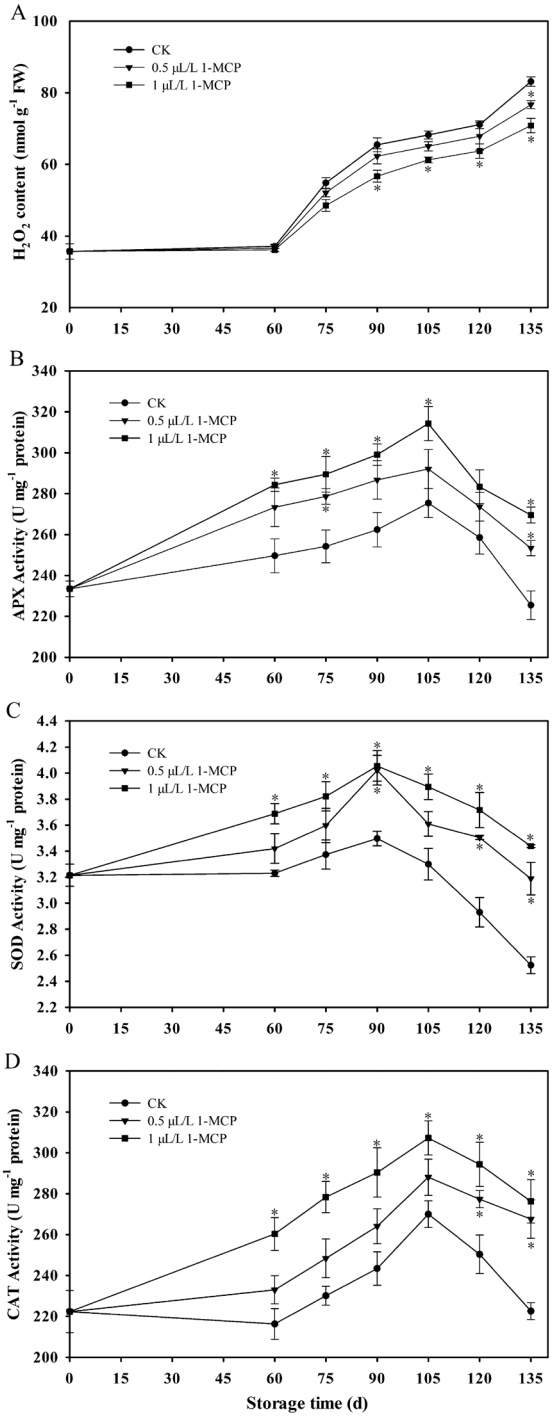

Fig. 3: Effects of 1-MCP on H_2O_2 content (A) and activities of APX (B), SOD (C), and CAT (D) in 'Wujiuxiang' pear fruits during storage at 0 °C. The data are the means ± SE of three replicates. The vertical bars represent the standard errors of the means. Star represents significant difference ($P < 0.05$).

Effects of 1-MCP on relative electrolyte leakage rate and LOX activity

The relative electrolyte leakage rate of the peel steadily increased in the control, reaching a value of 45.9% after 135 days of cold storage. At the same time, it also increased in the 1-MCP-treated fruit but to a much lesser degree. However, the difference between 0.5 µL/L 1-MCP-treated fruits and the control was not significant (Fig. 4A).

As shown in Fig. 4B, the LOX activities in the control fruit increased rapidly during 105 days of cold storage and then gradually decreased. In the 1-MCP-treated fruits, the LOX activities were maintained at a lower level during storage, and this was particularly true in the 1.0 µL/L 1-MCP-treated fruits.

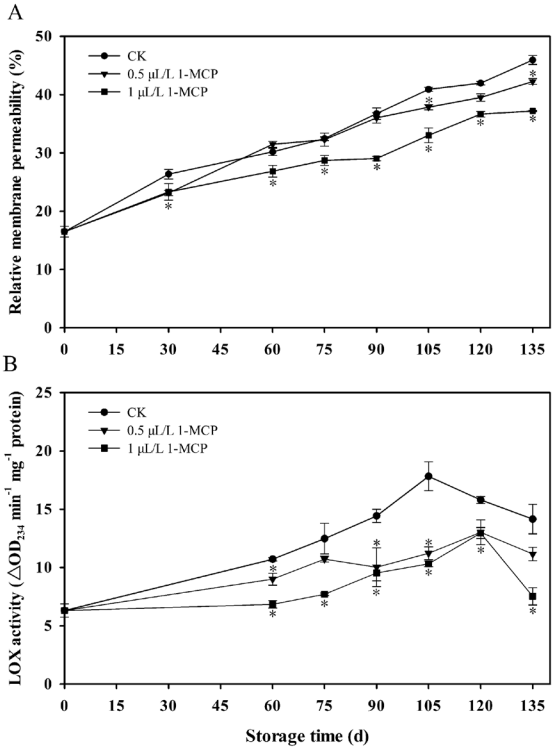

Fig. 4: Effect of 1-MCP on relative membrane permeability (A) and LOX activity (B) in 'Wujiuxiang' pear fruits during storage at 0 °C. The data are the means ± SE of three replicates. The vertical bars represent the standard errors of the means. Star represents significant difference ($P < 0.05$).

The correlation between H_2O_2 content and the cell membrane permeability of the peel, and between H_2O_2 content and scald index

To reveal the relationship between H_2O_2 content and the cell membrane permeability of the peel, and between H_2O_2 content and the scald index, the correlation between these variables was calculated. The finding showed a very significant positive correlation between the H_2O_2 content and the relative electrolyte leakage rate of the peel ($r = 0.881$, $P < 0.01$, Fig. 5), and a significant positive correlation between the H_2O_2 content and scald index ($r = 0.782$, $P < 0.05$, Fig 5).

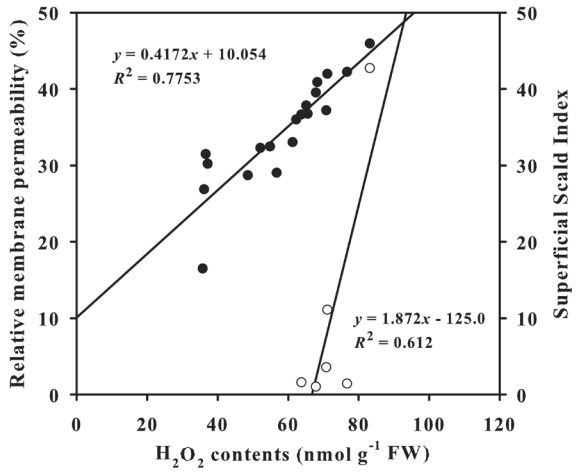

Fig. 5: Correlation between H_2O_2 content and the relative memebrane permeability of the peel (●), and between H_2O_2 content and scald index (○) in 'Wujiuxaing' pear.

Discussion

The plant hormone ethylene is considered not only the major signaling molecule that regulates most aspects of fruit ripening in climacteric fruit (Pech et al., 2012), but also an important regulator in disorder development during storage, including superficial scald (Lurie and Watkins, 2012). Owing to its effects on ethylene action, 1-MCP was applied to delay ripening, to maintain fruit quality during storage (Blankenship and Dole, 2003; Watkins, 2006) and to reduce physiological disorder, including superficial scald (Bai et al., 2009; Hui et al., 2011; Li and Wang, 2009; Sabban-Amin et al., 2011). In 'Wujiuxiang' pear, treatment with 1.0 µL/L 1-MCP also significantly reduced the superficial scald index, retained a higher firmness and a lower TSS content, and decreased the ethylene production and respiration rates (Fig. 1). This finding suggested that 1-MCP is effective for delaying fruit softening and inhibiting the incidence of superficial scald in 'Wujiuxiang' pear. Moreover, the 1-MCP concentration at 1.0 µL/L was more effective than that at 0.5 µL/L.

It was interesting that scald appeared at day 120 and become more serious at day 135 in control during cold storage (Fig. 1A). It suggested that scald appeared suddenly and developed quickly at late stage of cold storage in 'Wujiuxiang' pear, which was similar to 'Bartlett' pear (Whitaker et al., 2009). It may due to 'Wujiuxiang' pear is a hybrid cultivar from 'Yali' (*Pyrus bretschneideri* R.) and 'Bartlett' (*Pyrus communis* L.), and more like Europe pear (*Pyrus communis* L.) in postharvest storage characteristics.

In fruit, the inhibition of ethylene production by 1-MCP is well known. 1-MCP could almost fully inhibit ethylene production in 'Fuji' apple (Lu et al., 2013), 'Granny Smith' apple (Sabbban-Amin et al., 2011), and 'Delicious' apple (Apollo Arquiza et al., 2005); and delayed the increase of ethylene production in 'Bartlett' pear (Ekman et al., 2004), 'Anjou' pear (Argenta et al., 2003), and 'Conference' pear (Chiriboga et al., 2013b). In this work, compared to control, ethylene production in 1-MCP-treated 'Wujiuxiang' pear fruits showed similar pattern with a much lower concentration (Fig. 1E). It suggested that the 1-MCP could partially inhibit the ethylene action in 'Wujiuxiang' pears. Due to this, 1-MCP could not fully prevent scald development (Fig. 1A), nor did it preventing firmness loss (Fig. 1B), but both were less than in control. There are some possible reasons for the partial inhibition of 1-MCP on ethylene action. Firstly, it is clear that 1-MCP inhibits the ethylene responses by blocking the ethylene receptor with the highly stable 1-MCP-receptor complex (Sisler and Serek, 1997). However, treatment with 1-MCP prior to storage could only block existing receptors, but new ethylene receptors could be formed during storage (Tsantili et al., 2007). Previous results showed that the transcriptional levels of *PcETR1* and *PcETR2* were higher in 1-MCP-treated pear fruits than those in control during cold storage (Chiriboga et al., 2013b). It could be proposed that the inhibition effects of 1-MCP on ethylene

action were offset by newly translated ethylene receptors during cold storage. Secondly, the ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in different fruit cultivars (Dal Cin et al., 2006), harvest maturity and storage temperature (Chiriboga et al., 2013b), the details of the inhibition mechanism of 1-MCP on ethylene action in 'Wujiuxiang' pears require additonal experiments.

Ethylene can regulate α-farnesene accumulation in apples (Ju and Curry, 2000; Whitaker et al., 2000) and pears (Isidoro et al., 2006; Li et al., 2009). Our results also suggested that the accumulation of α-farnesene may be associated with ethylene production. The results showed that the productions of α-farnesene and ethylene both peaked at 60 day and that 1-MCP inhibited the productions of ethylene and α-farnesene (Fig. 1E and Fig. 2A). Furthermore, it has been shown that α-farnesene metabolism may be a manipulating point to control scald development. The reactive conjugated trienols resulting from the oxidation of α-farnesene are believed to be directly responsible for superficial scald (Du and Bramlage, 1993; Giné Bordonaba et al., 2013; Lu et al., 2013; Whitaker, 2007). In our experiment, the accumulation of α-farnesene in all fruits peaked at 60 days of storage, whereas the accumulation of conjugated trienols showed an increase trend at later storage period (Fig. 2), which indicates that the production of α-farnesene is followed by the accumulation of conjugated trienols. Thus, it further confirmed that superficial scald was more closely associated with conjugated trienols than with α-farnesene (Giné Bordonaba et al., 2013; Lu et al., 2013).

It has been suggested that ethylene plays a fundamental role in scald development not only by regulating the α-farnesene metabolism but also by regulating the anti-oxidant system in the fruit (Lu et al., 2013; Sabban-Amin et al., 2011). It is thought that high H_2O_2 concentrations, which accumulate in fruits during prolonged cold storage (Dong et al., 2011; Lu et al., 2011; Rao et al., 1998), are often regarded as an indication of oxidative stress (Apel and Hirt, 2004). H_2O_2 together with O_2^- may form the radical hydroxyl ·OH, which is a powerful oxidant. This product may cause many important metabolic changes, such as lipid peroxidation (Tian et al., 2013), which may result in damage to the cell membrane and physiological disorders, such as superficial scald (Lu et al., 2011, 2013; Rao et al., 1998; Sabban-Amin et al., 2011). The findings showed that the accumulation of H_2O_2 in peel is much lower in the 1.0 μL/L 1-MCP-treated fruits than that in control during cold storage (Fig. 3A). This effect was found to be associated with a lower relative electrolyte leakage rate (Fig. 4A) and a lower scald index (Fig. 1A), which reflected that the lipid peroxidation was reduced in the 1-MCP-treated fruit, and also, the lower H_2O_2 level was closely related to lower relative membrane permeability and lower scald index (Fig. 5). As another indicator of lipid degradation, LOX activity is closely correlated with lipid peroxidation during fruit ripening (Li and Wang, 2009; Liu et al., 2013; Singh et al., 2012). The data showed that the LOX activities were kept at a low level in the 1-MCP-treated fruits (Fig. 4B). Therefore, this finding suggested that 1-MCP can protect the membrane integrity by inhibiting H_2O_2 accumulation and lipid peroxidation.

In general, the H_2O_2 levels in plant cells are regulated by H_2O_2-scavenging enzymes, such as APX, SOD and CAT (Rao et al., 1996; Tian et al., 2013). The lower H_2O_2 levels observed in the peel of 'Wujiuxiang' pears treated with 1-MCP might be resulted from the higher activities of APX, SOD and CAT (Fig. 3B, C, D). These results are in agreement with previous findings in 'Blanquilla' pear (Larrigaudiere et al., 2004) and 'Granny Smith' apple (Sabban-Amin et al., 2011). All of the results suggested that 1-MCP can reduce H_2O_2 accumulation by improving the activities of H_2O_2-scavenging enzymes.

Previous research has indicated that the extent of oxidation of α-farnesene could be affected by anti-oxidant enzyme activities in fruit (Whitaker, 2004; Rao et al., 1998). In this work, the anti-oxidant enzyme activities were higher (Fig. 3B, C, D), and the conjugated trienols were lower (Fig. 2B) in the 1-MCP-treated fruits than those in the control. This finding suggested that 1-MCP inhibits the accumulation of conjugated trienols not only due to the lower α-farnesene accumulation (Fig. 2A) but also the reduced oxidation reaction, which may a result of the higher anti-oxidant enzyme activities and lower H_2O_2 levels (Fig. 3).

In conclusion, these data showed that 1-MCP delayed softening and inhibited scald development in 'Wujiuxiang' pear fruit. On the basis of these results and previous work, we propose the following possible mechanism for the inhibition of superficial scald by 1-MCP: By inhibiting ethylene synthesis and/or signal transduction, 1-MCP stimulates the activities of H_2O_2-scavenging enzymes to suppress the accumulation of H_2O_2, which results in the reduction of cell membrane damage and the lower accumulation of conjugated trienols, and thus inhibits cell death and superficial scald of peel. However, the crosstalk between H_2O_2 and α-farnesene metabolism remains unclear, and future work is needed.

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Address of the corresponding author:
E-mail: junfeng-guan@263.net