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## Different response to 1-methylcyclopropene in two cultivars of Chinese pear fruit with contrasting softening characteristics

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(Submitted: January 9, 2019; Accepted: February 14, 2019)

### Summary

In this study, the change in softening and its related genes expression under influence of 500 nL L<sup>-1</sup> 1-methylcyclopropene (1-MCP) was assessed in the two Chinese pear fruit, 'Jingbaili' (*Pyrus ussuriensis* Maxim) and 'Yali' (*Pyrus bretschneideri* Rehd), which exhibit different softening characteristics. 'Jingbaili' pear fruit softened rapidly after harvest, and was strongly inhibited by 1-MCP. In contrast, there was no obvious change of firmness compared to the control after 1-MCP treatment in 'Yali' pear fruit. The respiration and ethylene production rates were reduced by 1-MCP at early storage in both two cultivars. 'Jingbaili' pear fruit exhibited dramatically increased expression levels of the softening-related genes, i.e., polygalacturonase1 (*PG1*), polygalacturonase2 (*PG2*),  $\beta$ -Galactosidase4 (*GAL4*),  $\alpha$ -arabinofuranosidase1 (*ARF1*) and  $\alpha$ -arabinofuranosidase2 (*ARF2*), and these genes' expression levels were significantly decreased by 1-MCP treatment. In contrast, 'Yali' pear fruit showed lower expression levels of the above-mentioned genes, as well as a relatively smaller inhibition effect by 1-MCP treatment before day 27. These results suggest that 'Jingbaili' pear fruit are more sensitive to 1-MCP/ethylene than 'Yali' pear fruit during ripening.

**Keywords:** pear; 1-methylcyclopropene; softening; cell wall enzyme; gene expression

### Introduction

'Jingbaili' (*Pyrus ussuriensis* Maxim) and 'Yali' (*Pyrus bretschneideri* Rehd) pear fruit are famous cultivars and belong to typical Chinese pear with different softening and ripening behaviors. 'Jingbaili' pear fruit exhibits an extremely rapid decrease in flesh firmness during ripening, while 'Yali' pear fruit do not exhibit fruit softening and maintain a crispy texture even during the late stage of ripening (HIWASA et al., 2004; WEI et al., 2009, 2015).

1-methylcyclopropene (1-MCP), an effective inhibitor of ethylene action, has been found to delay softening of fruits, such as apple, banana, kiwifruit, mango, tomato and durian by blocking the ethylene signal transduction pathway, and has been widely used to investigate fruit tissue responses to ethylene during ripening and senescence of climacteric fruit (GOLDING et al., 1998; JIANG and JOYCE, 2002; GAMRASI et al., 2010; VILAS-BOAS et al., 2007; PIRIYAVINIT et al., 2011; LU et al., 2012; AMORPUTTI et al., 2014).

Cell wall disassembly is believed to contribute to fruit softening (WAKABAYASHI, 2000; SAÑUDO-BARAJAS et al., 2009). The depolymerization and solubilization of the pectin polymer is the major factor involved in fruit softening, and the pectin modification that cause cell dispersion and hydrolysis of the cell wall polymers is associated with texture change (BRUMMELL, 2006; GOULAO et al., 2008; GWANPUA et al., 2014). Many studies have shown that pectin modification is

caused by the action of a series of cell wall-modifying enzymes and proteins, such as polygalacturonase (PG),  $\beta$ -Galactosidase ( $\beta$ -GAL) and  $\alpha$ -arabinofuranosidase ( $\alpha$ -ARF) (BRUMMELL, 2006; GWANPUA et al., 2014; WEI et al., 2015). These enzymes have the capacity to reduce intercellular connections and the molecular size of pectin polymers by cleaving the backbone or the side chain residues. The modified polymers play different roles in fruit softening (SAÑUDO-BARAJAS et al., 2009; WEI et al., 2015).

In this study, we investigated the effects of 1-MCP on fruit softening of 'Jingbaili' and 'Yali' pears. Furthermore, we measured changes in the expression patterns of the softening-related genes (*PG1*, *PG2*,  *$\beta$ -GAL4*, *ARF1* and *ARF2*) to analyze the differences in the responses to 1-MCP between two cultivars of pear fruits.

### Materials and methods

#### Biological materials and treatments

'Jingbaili' (*Pyrus ussuriensis* Maxim.) and 'Yali' (*Pyrus bretschneideri* Rehd.) pear fruit were harvested at commercial maturity (For 'Jingbaili' pear fruit: Average weight: 101.7  $\pm$  5.8 g, TSS: 12.2  $\pm$  0.9%; For 'Yali' pear fruit: Average weight: 179.2  $\pm$  10.7 g, TSS: 10.9  $\pm$  1.0%) (Sep. 15, 2014) from an orchard in Changli County, Hebei Province, China. The fruit without mechanical injury, insects and diseases were placed directly in a sealed container with 500 nL L<sup>-1</sup> 1-MCP (0.18%, Ansip<sup>®</sup>, Taiwan, China), After fumigating for 24 h at 20  $\pm$  1  $^{\circ}$ C, the fruit were then ventilated and stored at 20  $\pm$  1  $^{\circ}$ C. Control fruit were subjected to the same protocol with the exception of a lack of 1-MCP treatment in the sealed container. Fruit were sampled at appropriate intervals based on their softening rates, the flesh tissues were frozen in liquid nitrogen and stored at -70  $^{\circ}$ C for subsequent analysis.

#### Determination of fruit firmness, respiration rate, ethylene production rate

Flesh firmness was determined using a digital fruit penetrometer (TuoPu Instruments, Model GY-4, Zhejiang, China) with ten fruit per sampling time, and three replications per treatment. The respiration and ethylene production rates were measured via the air-stream method with three replications per treatment. Ethylene production rate was determined by using a gas chromatograph (Lunan Ruihong Chemical Instruments, Model GC-SP-6890, Shandong, China). 2.0 kg of fruit were placed in a 9.5 L airtight jar for 1 h at 20  $\pm$  1  $^{\circ}$ C. The 60  $\mu$ L of gas samples were withdrawn through a septum on top of the jar using a gas-tight syringe, and three replications per treatment.

#### RNA extraction and qRT-PCR analysis

Total RNA was extracted from the pear fruit samples using the modified CTAB method (GASIC et al., 2004). Based on our previous research (WEI et al., 2015), we choose *PG1*, *PG2*, *GAL4*, *ARF1*

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and *ARF2* to be measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). First-strand cDNAs were synthesized from DNase-digested RNA (0.5 µg) using the Takara RNA PCR Kit (AMV) Version 3.0 (TaKaRa Biomedicals, Japan). qRT-PCR was performed using the SYBR Premix Ex Taq™ (Perfect Real Time) Kit (TaKaRa Biomedicals, Japan) on a 7500 Real-Time PCR system (Applied Biosystems, USA). qRT-PCR primers for *PGs*, *GAL4* and *ARFs* have been reported by WEI et al. (2015). The qRT-PCR reaction was performed in a final volume of 25 µL containing 12.5 µL SYBR Green PCR Premix Ex Taq™, 1 µmol L<sup>-1</sup> forward and reverse primers, and 10 ng cDNA with cycles as follows: 10 s at 95 °C, 40 cycles of 95 °C for 5 s, and 60 °C for 34 s. The T<sub>m</sub> of the amplification products was analyzed using a dissociation curve to confirm the quality of the PCR product and primer specificity. *Actin2* (*ACT2*) was used as a reference gene. All qRT-PCR reactions were normalized using Ct value corresponding to the *Actin2* gene. The relative expression levels of the detected genes were calculated with the formula  $2^{-\Delta\Delta CT}$  and the experiment was performed with four replicates per treatment.

### Statistical analysis

The results were subjected to ANOVA statistically analyzed using SPSS 18 software (SPSS Inc., Chicago, IL, USA). All of the values are expressed as the means ± SD of three replicates. Least significant differences (LSD) at a significance level of 0.05 were generated by ANOVA.

## Results

### Respiration and ethylene production rates and flesh firmness

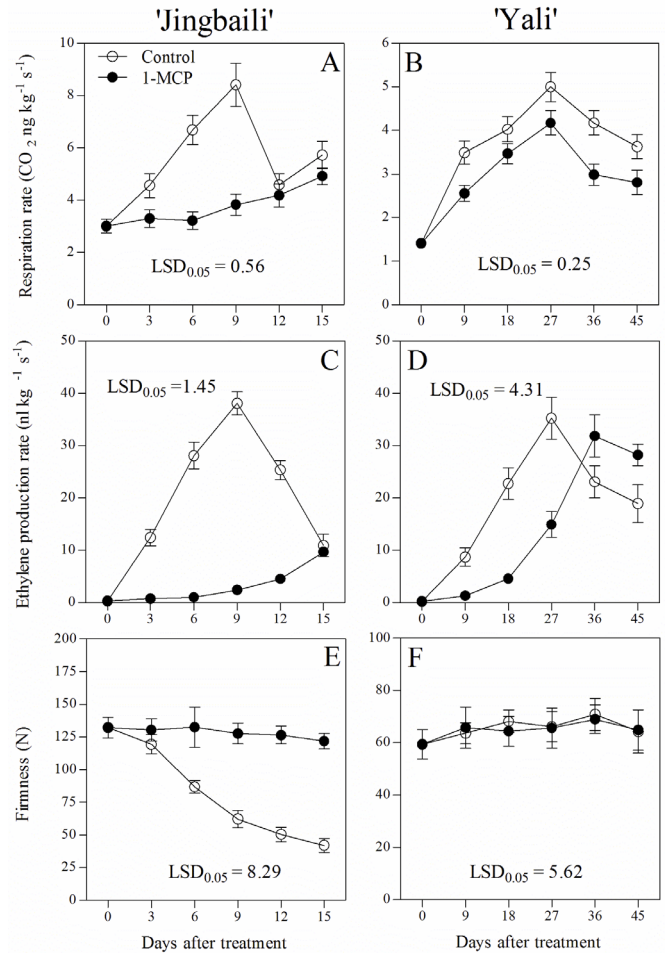
In 'Jingbaili' pear fruit, the respiration and ethylene production rate reached their peaks at the day 9 of storage concomitant with a rapid decrease in flesh firmness during ripening. After exposure to 1-MCP, the respiration and ethylene production rates dramatically decreased at first. And moreover, their climatic peaks were all delayed, and flesh firmness was noticeably kept at an initial value (Fig. 1 A, C and E). In contrast, although 1-MCP resulted in a decrease trend in the respiration during storage, as well as the ethylene production rates before day 27 of storage in 'Yali' pear, it did not exhibit the marked loss of flesh firmness (Fig. 1 B, D and F).

### PG gene expression

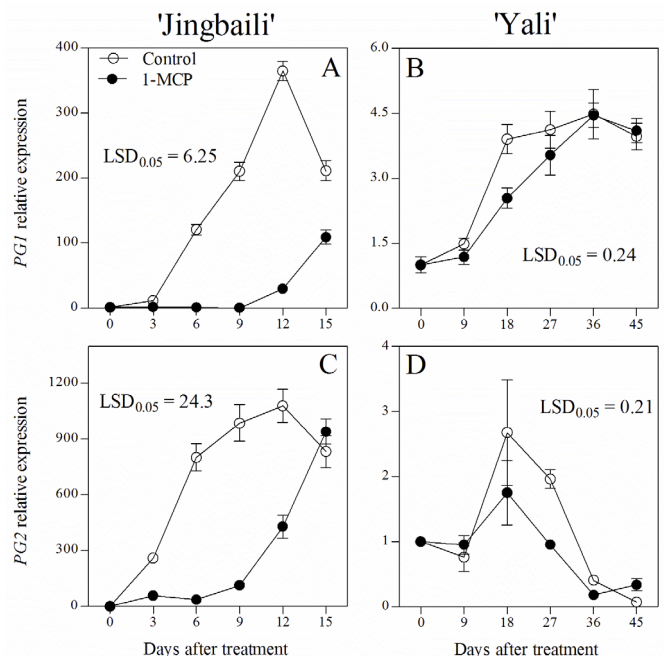
Both the *PG1* and *PG2* transcripts were expressed at very high levels and were rapidly enhanced before day 12 of storage in 'Jingbaili' pear fruit, and both of the *PG1* and *PG2* mRNA accumulated slowly and were dramatically reduced in 1-MCP treatment than in control, except at day 15 for *PG2* (Fig. 2 A and C). Relatively, the mRNA amounts of *PG1* and *PG2* were much lower in 'Yali' pear than that in 'Jingbaili' pear, and the peaks were found at day 36 for *PG1* and day 18 for *PG2*. In addition, *PG1* and *PG2* were significantly decreased by 1-MCP at day 18 and day 27, respectively (Fig. 2 B and D).

### β-GAL gene expression

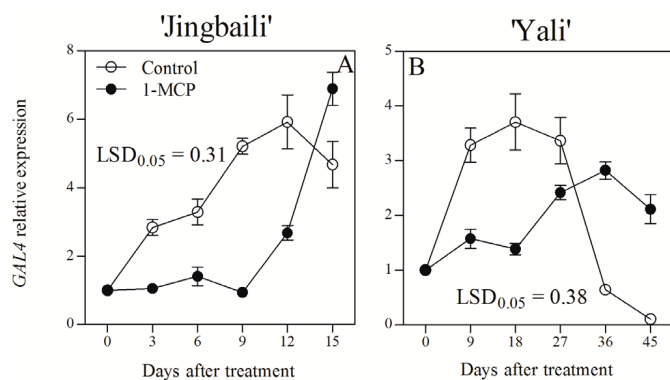
The *GAL4* is the major softening-related gene involved in fruit ripening, consistent with the changes in β-GAL activity in pear fruit (WEI et al., 2015). Therefore, the expression level of *GAL4* was studied here. The mRNA of *GAL4* accumulated rapidly and was dramatically inhibited by 1-MCP in 'Jingbaili' pear before day 12 of storage (Fig. 3A). The expression of *GAL4* in 'Yali' pear rose to a peak at day 18 in control, meanwhile, it increased slowly before day 27 of storage and afterwards reached the peak at day 36 in 1-MCP treatment (Fig. 3B).



**Fig. 1:** Effects of 1-MCP on the rates of respiration (A and B) and ethylene production (C and D), and firmness (E and F) in 'Jingbaili' (A, C and E) and 'Yali' (B, D and F) pear fruit. Values are the means ± SD.



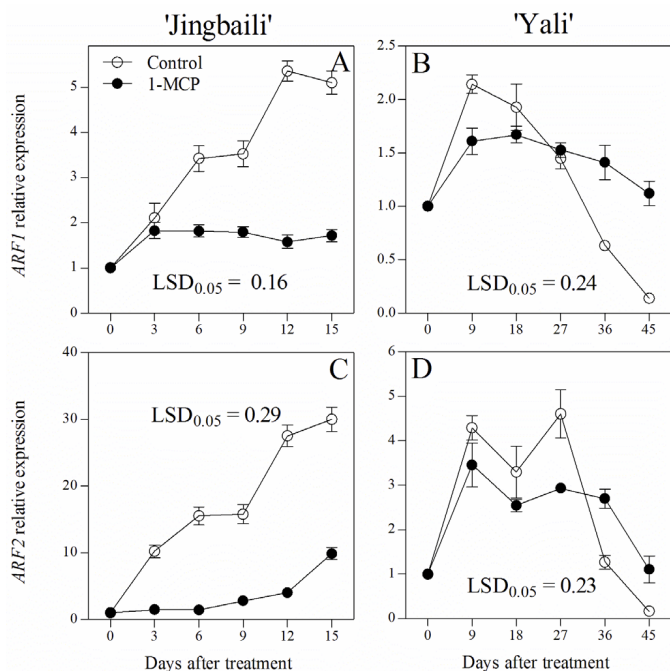
**Fig. 2:** Effect of 1-MCP on the expression of *PGs* in 'Jingbaili' (A and C) and 'Yali' (B and D) pear fruit. Values are the means ± SD.



**Fig. 3:** Effect of 1-MCP on the expression of *GAL4* in 'Jingbaili' (A) and 'Yali' (B) pear fruit. Values are the means  $\pm$  SD.

### $\alpha$ -ARF gene expression

The expression level of *ARF2* in 'Jingbaili' pear was higher and increased more rapid than that of *ARF1*, while the expression levels of the *ARF1* and *ARF2* genes increased slowly and showed much lower levels in 1-MCP treatment than that in control (Fig. 4A and C). However, both *ARF1* and *ARF2* expression were lower and there was a lower level of *ARF1* expression at day 9 and 18, and *ARF2* at day 9, 18 and 27, even though there was a higher expression level of *ARF1* in 1-MCP treatment during the later stage in 'Yali' pear fruit (Fig. 4B and D).



**Fig. 4:** Effect of 1-MCP on the expression of *ARFs* in 'Jingbaili' (A and C) and 'Yali' (B and D) pear fruit. Values are the means  $\pm$  SD.

### Discussion

1-MCP can effectively inhibit fruit tissue responses to ethylene, thereby depress respiration, ethylene production and delaying ripening of climacteric fruit (SEREK et al., 1995). Therefore, the application of 1-MCP provides an alternative approach to assess the ripening-related mechanism in many fruits. The maintenance of better flesh texture and effective delay in fruit softening by 1-MCP treatment has been widely reported in many fruits such as plum (LUO

et al., 2009; MINAS et al., 2013), mangosteen (PIRIYAVINIT et al., 2011), apple (YANG et al., 2013; IRELAND et al., 2014), pear (XIE et al., 2014; ESCRIBANO et al., 2017), tomato (DONG et al., 2013) and muskmelon (SUPAPVANICH et al., 2013). In this study, application of 1-MCP after harvest dramatically delayed softening in 'Jingbaili' pear fruit but had little effect in 'Yali' pear fruit (Fig. 1E and F), suggesting that 'Jingbaili' pear is more sensitive to ethylene for regulating fruit softening than 'Yali' pear.

An increase in PG activity and mRNA level has been reported in several fruit species concomitant with the degradation of pectin polysaccharides and softening (HIWASA et al., 2003, 2004; WEI et al., 2015, SONG et al., 2016; GWANPUA et al., 2016). 1-MCP treatment restricted softening and reduced PG activity and the expression level of *PGs* genes in pear and papaya fruits after the onset of ripening (HIWASA et al., 2003, 2004; ZERPA-CATANHO et al., 2017). A similar result was observed in the 'Jingbaili' pear fruit, which both *PG1* and *PG2* expression presented at much higher levels in control and were sharply suppressed by 1-MCP during the onset of softening in the 'Jingbaili' pear fruit (Fig. 2A and C). By contrast, *PGs* expression in 'Yali' pear fruit was relatively less affected by 1-MCP treatment (Fig. 2B and D).

RANWALA et al. (1992) found that purified  $\beta$ -GAL had the capacity to catalyze an apparent decrease in the molecular size of pectins *in vitro*. In strawberry, down-regulated of *Fa $\beta$ -GAL4* increases cell wall galactose levels and reduces fruit softening (PANIAGUA et al., 2016). 1-MCP treatment inhibited mRNA accumulation of *CmGAL* in melon, suggesting that the expression of *CmGAL* is likely to be dependent on ethylene and may therefore function as a potential co-operative action partner with *PG1* (NISHIYAMA et al., 2007). In this study, we found that the expression of *GAL4* was dramatically inhibited by 1-MCP treatment in 'Jingbaili' pear fruit (Fig. 3A). In agreement with report that the expression of *GAL4* was negatively correlated with water- and  $\text{Na}_2\text{CO}_3$ -soluble polymers (rich in neutral sugars) in the 'Jingbaili' pear fruit (WEI et al., 2009). In contrast, the expression level of *GAL4* was maintained at a low level under 1-MCP treatment in 'Yali' pear fruit with a slower increase at first and then dropped (Fig. 3 B). It was in agreement with the results reported by MWANIKI et al. (2005) and TATEISHI et al. (2005).

The possible involvement of a glycoside hydrolase such as  $\alpha$ -ARF in cell wall modification during fruit ripening has been well documented. *ARF* expression could result in the release of in many fruits (SOZZI et al., 2002; BRUMMELL et al., 2004; NISHIYAMA et al., 2007; WEI et al., 2010). In 'Jingbaili' pear fruit, *ARFs* gene expression rapidly increased and were significantly inhibited by 1-MCP treatment (Fig. 4 A and C), while in 'Yali' pear fruit, they were maintained at a lower level and exhibited only a slight inhibition by 1-MCP (Fig. 4 B and D). These results suggested that *ARFs* in 'Jingbaili' pear fruit were more sensitive to ethylene than those in 'Yali' pear fruit. Furthermore,  $\alpha$ -ARF may play different roles in pear fruit softening due to the different textural attributes and response to ethylene as shown in tomato (SOZZI et al., 2002) and apple (GOULAO et al., 2007).

### Conclusion

In summary, this study indicated that the fruit softening was significantly inhibited by 1-MCP treatment in 'Jingbaili' pear, while no obvious change was observed in 'Yali' pear. This was caused by the different change of expression of the softening-related genes, i.e., *PG1*, *PG2*, *GAL4*, *ARF1* and *ARF2*. This suggests that the 'Jingbaili' pear is more sensitive to 1-MCP/ethylene than the 'Yali' pear during ripening.

### Acknowledgements

This work was supported by the emarked fund for the China Agriculture Research System for National Technology System for



Pear Industry (CARS-28-05B), the Innovation Project of Modern Agricultural Sciences and Technology of Hebei Province, China (494-0402-YSN-C8RA) and Finance Special Foundation of Hebei province (F18R060018-9).

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