Scientia Horticulturae 229 (2018) 157–166

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

# Effects of methyl jasmonate on expression of genes involved in ethylene biosynthesis and signaling pathway during postharvest ripening of apple fruit

Jingyi Lv*[,1], Mengyuan Zhang[1], Junhu Zhang, Yonghong Ge, Canying Li, Kun Meng, Jianrong Li*

*College of Food Science and Project Engineering, Bohai University; National & Local Joint Engineering Research Center of Storage, Processing and Safety Control Technology for Fresh Agricultural and Aquatic Products, Bohai University, Jinzhou, Liaoning 121013, PR China*

ARTICLE INFO

*Keywords:*
Apple fruit
Ethylene biosynthesis
Signal transduction
Jasmonates
Fruit ripening
Gene expression

ABSTRACT

Ethylene plays a key regulatory role in the ripening of climacteric fruit. In addition to ethylene, jasmonates (JAs) have also been demonstrated to play a role in the regulation of the fruit ripening. Apple (*Malus* × *domestica* Borkh.) fruit is a common climacteric fruit. Research has been conducted to illustrate the effects of JAs on ethylene production in apple fruit, but little is known about the molecular mechanisms underlying the role of JAs in ethylene biosynthesis and signaling pathway during the ripening of apple fruit. To better understand the effects of JAs on the expression of key genes involved in the ethylene biosynthesis and signaling pathway during postharvest ripening of apple fruit, apples harvested at commercial maturity were treated with methyl jasmonate (MeJA). Our data indicated that MeJA treatment increased ethylene production during fruit ripening. The expression of *MdACS1*, *MdACS6*, *MdETR1*, *MdCTR1-3*, *MdCTR1-4*, *MdCTR1-5*, *MdEIN2A*, *MdEIN2B*, *MdEIL4* and *MdERF1* was positively regulated by MeJA treatment at the early ripening stage, whereas the expression of *MdEIL3* was positively regulated by it at the late ripening stage. MeJA treatment enhanced the expression of *MdACS3a*, *MdACS8*, *MdACO1*, *MdACO2*, *MdETR2*, *MdERS1*, *MdERS2* and *MdEIL1* during the entire storage period, whereas it had no effect on the expression of *MdCTR1-1*, *MdCTR1-2* and *MdEIL2* during fruit ripening. The expression of *MdERF2* and *MdACS1* was negatively by MeJA treatment during the period when the ethylene peak existed. These results indicated that the expression of genes involved in the ethylene biosynthesis and signaling pathway was differentially regulated by JAs during postharvest ripening of apple fruit.

## 1. Introduction

The gaseous phytohormone ethylene plays critical roles in the regulation of many plant physiological processes, including seed germination, seedling growth, organ abscission, fruit ripening, and senescence (Abeles et al., 1992; Tatsuki, 2010). Apple fruit is a climacteric fruit, whose ripening is characterized by an exponential increase in ethylene production and respiration rate (Varanasi et al., 2013; Yang and Hoffman, 1984). Studies have confirmed that ethylene plays a key regulatory role in the ripening of apple fruit, such as flesh softening (Ireland et al., 2014), volatile biosynthesis (Yang et al., 2016) and anthocyanin accumulation (Whale and Singh, 2007). Biosynthesis of ethylene begins with methionine, which is converted to S-adenosyl-L-methionine (SAM) by SAM synthetase. SAM is then catalyzed by 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS) to produce ACC, which can be further converted to ethylene by ACC oxidase (ACO) (Adams and Yang, 1979). The formation of ACC is generally considered to be the rate-limiting step in the biosynthesis of ethylene (Kende, 1993). In apple, five *ACS* genes and two *ACO* genes have been found to be related to fruit ripening, including *MdACS1*, *MdACS3a*, *MdACS6*, *MdACS7*, *MdACS8*, *MdACO1* and *MdACO2* (Dal Cin et al., 2007; Li et al., 2013; Wiersma et al., 2007).

After synthesis, ethylene is perceived by receptors that act as negative regulators of ethylene responses in plants (Kevany et al., 2007). Binding of ethylene by receptors inhibits the activity of constitutive triple response 1 (CTR1), a Raf-like serine/threonine (Ser/Thr) kinase, and then promotes the cleavage of the carboxyl end of ethylene insensitive 2 (EIN2) (Kieber et al., 1993; Wen et al., 2012). The carboxyl end of EIN2 (CEND) is a trafficking molecule transported into the nucleus where the transcription factors ethylene insensitive 3 (EIN3) and its homolog ein3-like 1 (EIL1) initiate a transcriptional cascade involving ethylene response factor (ERF) and other ethylene response DNA-

* Corresponding authors.
*E-mail addresses:* jingyi.lv@foxmail.com (J. Lv), lijr6491@163.com (J. Li).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scienta.2017.11.007
Received 11 August 2017; Received in revised form 26 October 2017; Accepted 1 November 2017

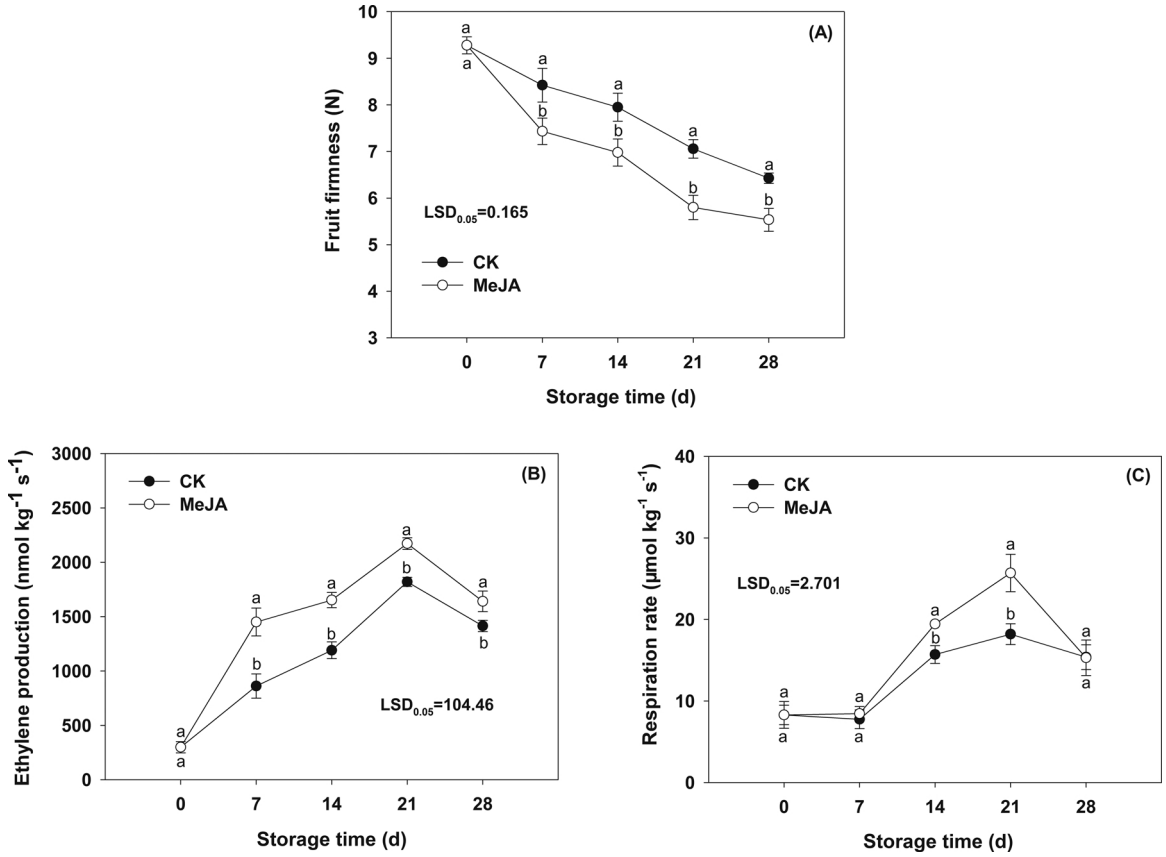


**Fig 1.** Fruit firmness, ethylene production and respiration rate of apple fruit during storage at 20 °C after treatment with 0.5 mM MeJA. Fruit firmness values were an average of 12 fruit with three measurements on each fruit. The ethylene production and respiration rate at each time point were calculated from the means of three biological replicates, and each replicate included three technical repeats. Vertical bars represent standard errors of means. The LSD (least significant difference) values were calculated at α-value of 0.05. Different letters above or below each data point indicate significant differences between treatment and control groups for each sampling day ($P < 0.05$).

binding factors (An et al., 2010; Chao et al., 1997; Ijaz, 2016). To date, six ethylene receptor genes (*MdETR1*, *MdETR1b*, *MdETR2*, *MdETR5*, *MdERS1* and *MdERS2*) have been reported in apple (Dal Cin et al., 2005; Tatsuki and Endo, 2006; Wiersma et al., 2007), and four of them (*MdETR1*, *MdETR2*, *MdERS1* and *MdERS2*) are considered to be related to fruit ripening (Li et al., 2010). Five splicing variants of *CTR1* (*MdCTR1-5*) have been investigated, and their expression was reduced by the ethylene antagonist 1-methylcyclopropene (1-MCP) during fruit ripening (Wiersma et al., 2007; Yang et al., 2013). Two *EIN2* genes (*MdEIN2A* and *MdEIN2B*), four *EIL* genes (*MdEIL1-4*) and two *ERF* genes (*MdERF1* and *MdERF2*) have now been isolated, and they were differentially expressed during the ripening of apple fruit (Wang et al., 2007; Wiersma et al., 2007; Yang et al., 2013).

Although ethylene is the major trigger for climacteric fruit ripening, other plant hormones, such as jasmonates (JAs) and abscisic acid (ABA), are shown to be tightly associated with fruit ripening (Kumar et al., 2014). The term jasmonates (JAs) is often used to refer to jasmonic acid, its volatilized methyl ester (methyl jasmonate, MeJA), and amino acid derivatives (Wasternack, 2007). Many studies have confirmed that exogenous JAs application can promote volatile emission (Kondo et al., 2005), stimulate anthocyanin synthesis (Rudell and Mattheis, 2008), accelerate chlorophyll degradation and β-carotene accumulation (Pérez et al., 1993). Fruit ripening is a complex process involving the dynamic interplay between different phytohormones. Previous studies have indicated a role for JAs in the regulation of ethylene biosynthesis during fruit ripening. In apples (Fan et al., 1997, 1998; Kondo et al., 2009) and pears (*Pyrus communis*) (Kondo et al., 2007), exogenous application of JAs at pre-climacteric stage may enhance ethylene emission, but opposite results were obtained when exogenous JAs were applied at climacteric and post-climacteric stage. By contrast, in peaches (*Prunus persica*), both early and late JAs treatment during fruit development resulted in the down-regulation of ethylene biosynthetic genes (Ruiz et al., 2013; Ziosi et al., 2008). In recent years, studies have shown that the expression of *MdACS1* in the skin of apple fruit was regulated by exogenous application of JAs (Kondo et al., 2009). However, the effects of JAs on the expression of ethylene biosynthetic genes during postharvest ripening of apple fruit are still unclear. By now, very few studies have been reported the effects of JAs on ethylene perception during fruit ripening. Only one report in peaches showed that the expression of ethylene receptor genes was differentially affected by exogenous application of JAs during fruit ripening (Ruiz et al., 2013; Soto et al., 2012). It is still unclear what is the effects of JAs on ethylene signaling pathway in ripening apple fruit. Therefore, the molecular mechanisms of JAs in the regulation of ethylene biosynthesis and signaling pathway during the ripening of apple fruit still need to be further elucidated. The aim of the present work was to investigate the effect of JAs on expression of key genes involved in the ethylene biosynthesis and signaling pathway during postharvest ripening of apple fruit.

## 2. Materials and methods

### 2.1. Plant materials and treatments

Apple fruit (*Malus* × *domestica* Borkh., 'Golden Delicious') without mechanical damage and free of visible defects or decay were harvested at commercial maturity from a commercial orchard in Jinzhou, China. Fruit were randomly sampled from over 80 individual trees and immediately transported to the postharvest laboratory of Bohai University. The selected fruit were randomly divided into two groups.