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Effect of 1-octylcyclopropene on physiological responses and expression of ethylene receptors gene in harvested tomato fruit

Fangxu Xu[a], Shiyang Liu[b], Xuqiao Feng[c,*]

[a] Experimental Teaching Center of Shenyang Normal University, Shenyang, Liaoning 110034, China
[b] Logistic Manage Office of Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, China
[c] Food Science Research Institute of Bohai University, Jinzhou, Liaoning 121013, China

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ABSTRACT

The aim of this study is to investigate the inhibitory effect of 1-octylcyclopropene (1-OCP), a structural analog of 1-methylcyclopropene (1-MCP), on postharvest ripening and senescence of tomato fruit (*Solanum lycopersicum* Mill., Shenyang, China). The results show that 1-OCP is effective in postponing the occurrence time of ethylene production peak and respiration rate peak, delaying the softening and color change, inhibiting the increasing of soluble solids and the decreasing of titratable acidity, and suppressing the expression of *LeETR* 1 and *LeETR* 4 in tomatoes. In addition, 1.2 $\mu L\,L^{-1}$ 1-OCP is found to be most effective in delaying ripening and senescence process of tomatoes by inhibiting the activities of enzymes involved in ethylene biosynthesis and gene expression of ethylene receptors. It is suggested that 1-OCP is potential as a given inhibitor when applied before the onset of ethylene action which is crucial when considering the promising candidates for practical use.

1. Introduction

As a typical climacteric fruit, fresh tomato is a perishable vegetable which senesces rapidly after harvest at ambient temperature. It is reported that ethylene plays a crucial role in the process of the ripening and senescence of tomato fruit (Bleecker and Kende, 2000). Ethylene production, respiration rates, color change and softening can be accelerated by endogenous ethylene (Sisler and Serek, 1997; Sisler and Wood, 1988). In addition to endogenous ethylene, exogenous ethylene can induce ripening-related changes, which seriously affects the storage and preservation of tomatoes. During the last decade, commercial product of 1-methylcyclopropene (1-MCP) appeared as a stable powder has been demonstrated to inhibit ethylene action (Cameron and Reid, 2001; Tian et al., 2000; Fan and Mattheis, 1999). The effects of 1-MCP on post-harvest physiological changes and quality of fruit and vegetables are extensive (Watkins, 2006). For example, 1-MCP can delay onset of ethylene climacteric for banana (Golding et al., 1998; Pathak et al., 2003), pear (Trinchero et al., 2004), tomato (Opiyo and Ying, 2005; Wang et al., 2010), apple (Fan and Mattheis, 1999), plum (Valero et al., 2003), persimmon (Luo, 2007) and avocado (Feng et al., 2000). However, the application of 1-MCP remains limited in agricultural practice because of the uneven color development of treated fruits. This problem is exacerbated because of the differences in the maturity ranges of the treated fruits presented in a commercial distribution (Cameron and Reid, 2001; Mir et al., 2001).

It is reported that structural analogs of 1-MCP, such as 1-ethylcyclopropene (1-ECP), 1-propylcyclopropene (1-PCP), 1-butylcyclopropene (1-BCP), 1-pentylcyclopropene (1-PentCP), 1-hexylcyclopropene (1-HCP), 1-heptylcyclopropene (1-HeptCP), 1-octylcyclopropene (1-OCP) and 1-decatylcyclopropene (1-DCP), elicit an effect similar to 1-MCP, that is, by irreversibly occupying the site of binding at the ethylene receptor level to inhibit ethylene action (Apelbaum et al., 2008; Feng et al., 2004; Sisler et al., 2003,1995). Ethylene action is achieved by regulating ethylene receptor and a series of signal transduction, and finally by controlling related genes expression (Sisler et al., 2003). The ethylene receptor gene family presently consists of six members *LeETR* 1, *LeETR* 2, *NR* (*LeETR* 3), *LeETR* 4, *LeETR* 5 and *LeETR* 6 in tomato fruit (Klee and Tieman, 2002). The receptor gene expression pattern of the six members is not all the same. *LeETR* 1 and *LeETR* 4 are at a higher expression level in all tomato tissues throughout development (Wei et al., 2005). Each receptor gene has a distinct pattern of expression throughout development and in response to external stimuli (Klee and Tieman, 2002). In tomato fruit, the expression of *LeETR* 1 in fruit peel is not very noticeable with the change of fruit development process. *LeETR* 1 is expressed

* Corresponding author at: Food Science Research Institute of Bohai University, No. 19 Keji Road, Taihe District, Jinzhou, Liaoning 121013, China.
E-mail address: feng_xq@hotmail.com (X. Feng).

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constitutively and not affected by ethylene, silver ion and auxin. In Arabidopsis, the increased ethylene responses of *ETR* 1 loss-of-function mutant indicated that *ETR* 1 may play more prominent role than the other receptors in ethylene signal transduction (Chang et al., 1993). Some expected and unexpected phenotypes related to ethylene response resulted from the ethylene receptor gene *LeETR* 1 transgenic tomato and reduced expression of *LeETR* 1 in tomato fruit. The transcription characteristics of *LeETR* 2 are similar with *LeETR* 1 in most tissues of tomato fruit, which is also constitutively expressed. But the expression of *LeETR* 1 is about five-fold that of *LeETR* 2 (Wei et al., 2005).

However, the expression patterns of the other four genes are highly regulated (Tieman and Klee, 1999). The expression levels of *NR* and *LeETR* 4 are relatively high in mature tomato fruit, especially *LeETR* 4 which accountes for about 50% of the total expression. The antisense tomato plant of *LeETR* 4 presents ethylene constitutive reaction which is mainly manifested in leaves grow up, petals fall off and fruit mature early etc. Loss of *LeETR* 4 results in dramatic morphological changes associated with increased ethylene responsiveness (Solano et al., 1998). *LeETR* 4 and *LeETR* 5 are induced by fruit ripening and organ senescence, and are at a higher level in tomato meristem, especially in mature flowers. These results indicate that *LeETR* 4 may play mediation role in ripening and senescent of tomato fruit.

This study mainly focused on the potency of 1-OCP which exhibits relatively maximum length of 1-position side chain of the main cyclopropene structure. The aim of the present work was to assess the ability of 1-OCP to control ripening and expression of *LeETR* 1 and *LeETR*4 in post-harvest tomato fruit, which provide an alternative potential ethylene inhibitor for production practice

2. Materials and methods

2.1. Fruit and treatments

Whole mature green tomatoes (*Solanum lycopersicum* Mill., Shenyang, China) without any treatment were purchased from a local orchard in Shenyang, China during the commercial harvesting season. Then the tomatoes were immediately transported to the laboratory of the College of Food Science, Shenyang Agricultural University on the same day. Damaged tomatoes and outliers in size and color were excluded and randomly divided into seven groups. Each group contained 60 tomatoes (each tomato 0.15–0.20 kg). At 2 h after purchase, these seven selected groups with 60 tomatoes each were separately sealed in seven plastic tents (50 cm × 50 cm × 50 cm) and the tomatoes were treated as follows: (1) untreated; (2) with 0.4 $\mu L\,L^{-1}$ of 1-OCP for 20 h; (3) with 0.8 $\mu L\,L^{-1}$ of 1-OCP for 20 h; (4) with 1.2 $\mu L\,L^{-1}$ of 1-OCP for 20 h; (5) with 0.4 $\mu L\,L^{-1}$ of 1-MCP for 20 h; (6) with 0.8 $\mu L\,L^{-1}$ of 1-MCP for 20 h; or (7) with 1.2 $\mu L\,L^{-1}$ of 1-MCP for 20 h. All treatments were conducted at 20 ± 2 °C, RH 80–85%. After treatment, half of the tomatoes from the seven vented plastic tents each were stored in ambient conditions (20 ± 2 °C; RH 80–85%). Ripening was assessed by determining ethylene production, respiration rate, firmness, color change, soluble solids and titratable acidity every 5 d. The remaining half of the tomatoes were cut into small pieces (2 cm × 2 cm × 2 cm) and immediately frozen in liquid nitrogen, and stored at −80 °C for the analysis of gene expression of ethylene receptors (*LeETR* 1 and *LeETR* 4) every 5 days. Five tomatoes were taken from each group at each sampling time. Three measurements for each sample were performed and average value is reported.

2.2. Measurements of ethylene production and respiration rate

Tomatoes were sealed in ten 3-L glass jars (five fruit per jar) for 1 h at 20 °C. The ethylene production rate (ng $kg^{-1}\,s^{-1}$) was measured by withdrawing two gas samples (1 mL) with a gas syringe from each jar through a septum stopper fitted in the jar lid (Feng et al., 2000). Ethylene production was analyzed by a gas chromatograph (CP-3800 GC, Varian, USA) equipped with a flame ionization detector (FID) and a stainless steel column (1 m × 0.4 mm, GDX-502, Agilent, USA). The chromatographic analytical parameters were as follow: 60 °C, column temperature; 270 °C, detector temperature; and 0.06 mol s^{-1}, flow rate of N_2. The jars were left open between measurements for ventilation.

The respiration rate (ng $kg^{-1}\,s^{-1}$) was measured as CO_2 production. Tomatoes from each treatment were enclosed in a chamber for 5 min and air was passed through the chamber. The effluent air was connected to an Infrared Gas Analyzer (Li-840, Li-Cor, USA) and the respiration rate was measured.

2.3. Assessment of firmness and color change

Fruit firmness expressed in Newtons (N) was determined using a digital penetrometer (FT-327, Fruit Test™, Italy) fitted with a 5 mm diameter conical probe. This conical probe was pushed into the tomato through the skin to the depth of the head (10 mm) (Tian et al., 2000).

Tomato peel color was measured on three points of the fruit surface; on the shoulder, at the equator, and at the base. Color was measured using a portable Minolta Chroma Meter (Minolta, Japan) with LCH model calibrated with standard white plate (CY = 93.8, $x = 0.3164$, $y = 0.3208$). Color was expressed in coordinates *a*, *b* and *L*. Three readings from each fruit were averaged prior to data analysis (McGuire, 1992).

2.4. Measurement of soluble solids and titratable acidity

Tomatoes fruit were juiced individually using a blender (RW20, IKA, Germany). Fifty grams of flesh sample were diluted in 50 mL of distilled water and blended for 1 min until homogenized. The juice was further centrifuged at 6000 × *g* for 6 min (TGL-18C, Feige, China). A drop of supernatant from each sample was placed onto a refractometer (Atago, Japan) to measure the soluble solid content (%). This value was corrected for sample dilution to give the final soluble solid content (Liew and Lau, 2012).

Titratable acidity, determined by titration of the compost homogenized powder 10 mL sample with 0.1 M NaOH using fenolftaleine-indicator (expressed as% citric acid).

2.5. RNA extraction and cDNA preparation

Total RNA was extracted from tissues of frozen tomatoes by improved Guanidine Thiocyanate method according to Sambrook et al. (1989). Reverse transcription (RT) was conducted as follows according to the instructions of ReverTra Ace® (TOYOBO, Japan). RNA template (4 μL), 5 × first strand buffer (4 μL), 10 mM dNTPs (1 μL), 50 μM Oligo (dT) (1 μl), MMLT reverse transcriptase (ReverTra Ace®, TOYOBO, Japan) (1 μL) and DEPC treated water (8.5 μL) were mixed in 0.2 mL eppendorf tubes at 30 °C for 10 min, 42 °C for 60 min and 4 °C for 5 min. The tubes were immediately put into ice for 5 min. The products of RT were stored at −20 °C for future use.

Table 1
Sequences of primers and probes for quantitative analysis of genes expression with real-time PCR.

Name	Accession number	Gene ID	Sequence
LeETR 1	AF043084.1	606298	5′CGGATGAATCGCCTTT3′
LeETR 4	AF118843.1	543588	5′TCCTGAGTCCGAATAATACCC3′ 5′TGGTTGTAATGGCAGTCT3′ 5′ATCAGCAGCCGATAAGGAA3′