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Research Paper

Ethylene-influenced development of tree peony cut flowers and characterization of genes involved in ethylene biosynthesis and perception

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ABSTRACT

Effect of ethylene and ethylene antagonist 1-methycyclopropene (1-MCP) on postharvest development of two *Paeonia suffruticosa* cultivars was investigated. According to the monitoring results, postharvest development of 'Luoyang Hong' was accelerated by ethylene and postponed by 1-MCP treatment, while 'Xue Ying Tao Hua' was ethylene-insensitive. Ethylene production was elevated by ethylene treatment in 'Luoyang Hong', but our study failed to provide evidence for involvement of the isolated biosynthetic genes in ethylene response.

Three ethylene receptor genes were isolated and clustered with *AtETR1*, *AtERS1*, *AtEIN4*, respectively. Expression patterns of genes involved in ethylene signal pathway were tested during development of the two cultivars and in response to exogenous ethylene and 1-MCP. Expression patterns of *PsERS1* and *PsEIN4* showed significant differences between 'Luoyang Hong' and 'Xue Ying Tao Hua'. Besides, *PsEIL2*, *PsEIL3* and *PsERF1* may play a role in regulation of ethylene response of tree peony. The present study provides insights into ethylene biosynthesis and signal transduction in tree peony cut flowers. However, further studies were needed to verify the gene function and to understand the mechanism underlying postharvest development of tree peony.

1. Introduction

The gaseous plant hormone ethylene plays an important role in regulation of various physiological and developmental processes (Abeles et al., 2012) and has been extensively investigated in flower senescence process (Woltering and Van Doorn, 1988; Van Doorn, 2001, 2002). Genes encoding 1-aminocyclopropane-carboxylase (ACC) synthase (ACS) and ACC oxidase (ACO), the key enzymes involved in the well-defined ethylene biosynthetic pathway, are early targets of transgenic breeding programs aiming at better vase life of ornamental plants such as *Dianthus caryophyllus* (Savin et al., 1995; Kosugi et al., 2002), *Torenia fournieri* (Aida et al., 1998) and *Petunia hybrida* (Huang et al., 2007). Evolution of ethylene is highly regulated in response to endogenous or exogenous stimuli (Yang and Hoffman, 1984; Wang et al., 2002), and regulation of *ACS* and *ACO* genes occurs at both transcriptional level (Wang et al., 2002; Ruduś et al., 2013) and posttranscriptional level (Liu and Zhang, 2004; Argueso et al., 2007).

Aside from the amount of present ethylene, the way in which ethylene influences the senescence of cut flowers depends on the sensitivity of the floral organs, which helps to predict the negative effects of storage and transport, as well as usefulness of anti-ethylene treatments (Serek et al., 2006; Paul et al., 2012; Scariot et al., 2014). However, there is a lack of knowledge about the finite regulation mechanism determining ethylene sensitivity of cut flowers. Progress in understanding the regulation mechanism of ethylene response in plants has been achieved mainly by molecular studies on *Arabidopsis* and tobacco, and a linear signal transduction model has been established, according to which the ethylene signal is perceived by specific receptors and transmitted by a series of downstream components (Guo and Ecker, 2004; Chen et al., 2005). In *Arabidopsis*, five members of ethylene receptors are divided into subfamily 1 (AtETR1 and AtERS1) and subfamily 2 (AtETR2, AtERS2 and AtEIN4). These endoplasmic reticulum-localized proteins share high similarity with bacterial two-component histidine kinases and function collectively in ethylene response as negative regulators. In the presence of ethylene, the receptors fail to directly

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activate CTR1, the Raf-like serine-threonine kinase inhibiting signal transduction to the central regulator of the pathway, EIN2, and consequently activate EIN3/EILs and then ERF transcription factors, initiating ethylene responses (Merchante et al., 2013).

As the first components to receive and transmit ethylene signal, ethylene receptors have received the most attention in developing strategies to block the ethylene response in cut flowers. Prodigious progress has been made in exploring chemical inhibition and genetic manipulation of ethylene perception at the receptor level (Serek et al., 2006). However, studies have not reached a conclusion about the great variability of ethylene sensitivity between species and cultivars through studies on transcriptional regulation of ethylene receptor genes yet (Müller et al., 2000; Narumi et al., 2005; Tan et al., 2006). *CTR* genes, encoding the proteins involved in ethylene signal pathway via an unclear mechanism, showed different expression patterns during development and in response to ethylene in different species (Kieber et al., 1993; Leclercq et al., 2002; Ma et al., 2006). As to *EIN3* and *ERF* transcription factor genes, encoding the mediators of the cross-talk between various endogenous and exogenous signals, also exhibited complex expression patterns during opening and senescence of cut flowers (Rogers, 2013). Role of these components in regulating ethylene responses in more species is therefore worthy of investigation.

Tree peony (*Paeonia suffruticosa*), a famous traditional flower in China, is becoming increasingly popular around the world for its high esthetic value. Unfortunately, the short vase life of cut flowers hinders the development of tree peony industry. Our previous study has reported differences in ethylene sensitivity among cultivars within this species (Guo et al., 2003; Jia et al., 2009). However, there has been no systematic attempt to relate the effect of external ethylene on different cultivars to the response of genes involved in ethylene biosynthesis and perception.

In the present study, we chose two cultivars, 'Luoyang Hong' and 'Xue Ying Tao Hua', to further investigate the influence of exogenous ethylene and the competitive ethylene antagonist 1-methycyclopropene (1-MCP), and the transcriptional regulation of genes involved in ethylene biosynthesis and signal transduction in tree peony cut flowers, which would be of great value for understanding its senescence mechanism, as well as developing high-efficiency postharvest technology.

2. Materials and methods

2.1. Plant materials

Rose type cultivars *P. suffruticosa* 'Luoyang Hong' and 'Xue Ying Tao Hua' flowers were grown under field conditions in Heze, Shandong Province, China. According to our previous study (Guo et al., 2003), developmental stages of tree peony flowers were described as below: stage 1 (S1), soft bud stage; S2, pre-opening stage; S3, initial opening stage; S4, half opening stage; S5, full opening stage and S6, wilting stage.

Flowers were harvested at S1 with ~30 cm length of stems and the uppermost compound leaf intact, and then transported to the Lab of Flower Physiology and Application in Beijing Forestry University, Beijing, China, within 12 h. After the stems were trimmed under water to a length of 25 cm and all leaves were removed, cut flowers were rehydrated in distilled water for 1 h before further processing.

2.2. Treatments and sampling

After water recovery, middle petals (the 4th–6th layer petal) of three single cut flower replicates at S1 were carefully collected, immediately frozen in liquid nitrogen and stored at −80 °C until used for RNA extraction.

At the same time, treatments were conducted as follow: flowers at S1 with the stem ends placed in 0.05% (v/v) NaClO solution were incubated in a glass chamber with 10 $\mu L\,L^{-1}$ ethylene, 1 $\mu L\,L^{-1}$ 1-MCP or regular air (control), respectively, at room temperature (23–25 °C) for 6 h. After treatment, flowers were kept in the vase room at 23–25 °C with 50–60% relative humidity (RH), an illumination of ~40 $\mu mol\,m^{-2}\,s^{-1}$. 0.05% (v/v) NaClO solution was replaced every 24 h. During vase life, sampling of flowers at later developmental stages (S2–S6) was carried out in the same way as mentioned above for flowers at S1.

2.3. Evaluation of postharvest flower development

Five single cut flower replicates were used for evaluation of development.

Periodic visual inspections were carried out by the same person. Appearance of both cultivars were recorded more intensively during the early period at 0 h, 18 h and 36 h after treatment, and then the recording time was increased to every 24 h.

Flower diameter and fresh weight were measured every 6 h from the start of incubation (−6 h) and expressed as flower diameter changing rate and fresh weight changing rate, respectively, based on the following formulas:

$$\text{Flower diameter changing rate } (\%) = (D_n - D_{-6}) \times 100/D_{-6}$$

D_n is the flower diameter at n h after treatment and D_{-6} at the beginning of incubation.

$$\text{Fresh weight changing rate } (\%) = (W_n - W_{-6}) \times 100/W_{-6}$$

W_n is the fresh weight of flowers at n h after treatment and W_{-6} at the beginning of incubation.

Vase life was determined as the duration from the end of treatment to dramatic wilting and abscission of petals, when flowers finally lost their ornamental value.

Ethylene sensitivity was evaluated by the changes of vase life in response to the treatments.

2.4. Measurement of ethylene production

Three biological replicates were used for measurement of ethylene production. Individual flowers of each stage (S1-S6) were weighed and sealed in 4.75 L air-tight jars with a rubber stopper at room temperature for 1 h, then a 2 mL headspace gas sample was withdrawn using a hypodermic syringe, and the ethylene concentration was measured with a gas chromatograph GC-9900 (Jiafen, Beijing, China) with an alumina column and a flame ionization detector.

2.5. Total RNA extraction and cDNA synthesis

Frozen petals were ground in liquid nitrogen for total RNA isolation according to a reported method (Chang et al., 1993), and then digested with RNase-free DNase I (Takara, Dalian, China) to eliminate the potential DNA contamination. RNA integrity was assessed with 1.0% agarose gel electrophoresis and only RNA samples with 28S and 18S rRNA bands at a density ratio of 2:1 were used for further analysis. First-strand cDNA was synthesized from DNA-free RNA using oligo (dT) primer and Reverse Transcriptase