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RESEARCH PAPER

Expression of ethylene biosynthetic and receptor genes in rose floral tissues during ethylene-enhanced flower opening

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Abstract

Ethylene production, as well as the expression of ethylene biosynthetic (*Rh-ACS1–4* and *Rh-ACO1*) and receptor (*Rh-ETR1–5*) genes, was determined in five different floral tissues (sepals, petals, stamens, gynoecia, and receptacles) of cut rose (*Rosa hybrida* cv. Samantha upon treatment with ethylene or the ethylene inhibitor 1-methylcyclopropene (1-MCP). Ethylene-enhanced ethylene production occurred only in gynoecia, petals, and receptacles, with gynoecia showing the greatest enhancement in the early stage of ethylene treatment. However, 1-MCP did not suppress ethylene production in these three tissues. In sepals, ethylene production was highly decreased by ethylene treatment, and increased dramatically by 1-MCP. Ethylene production in stamens remained unchanged after ethylene or 1-MCP treatment. Induction of certain ethylene biosynthetic genes by ethylene in different floral tissues was positively correlated with the ethylene production, and this induction was also not suppressed by 1-MCP. The expression of *Rh-ACS2* and *Rh-ACS3* was quickly induced by ethylene in gynoecia, but neither *Rh-ACS1* nor *Rh-ACS4* was induced by ethylene in any of the five tissues. In addition, *Rh-ACO1* was induced by ethylene in all floral tissues except sepals. The induced expression of ethylene receptor genes by ethylene was much faster in gynoecia than in petals, and the expression of *Rh-ETR3* was strongly suppressed by 1-MCP in all floral tissues. These results indicate that ethylene biosynthesis in gynoecia is regulated developmentally, rather than autocatalytically. The response of rose flowers to ethylene occurs initially in gynoecia, and ethylene may regulate flower opening mainly through the *Rh-ETR3* gene in gynoecia.

Key words: Cut roses, ethylene biosynthesis, ethylene receptor, floral tissues, flower opening, gene expression, *Rosa hybrida*.

Introduction

Ethylene is one of the most important plant hormones, and plays a central role in various plant developmental processes, such as seed germination, flower and leaf senescence and abscission, and fruit ripening (Abeles *et al*., 1992). It also acts as an important modulator of plants responses to biotic and abiotic stimuli, including pathogen attack, flooding, chilling, and mechanical damage (Johnson and Ecker, 1998; Bleecker and Kende, 2000).

Ethylene biosynthesis in higher plants has been well defined (Yang and Hoffman, 1984). The first committed step of ethylene biosynthesis is the conversion of *S*-AdoMet to ACC by ACC synthase (ACS). ACC is then oxidized to ethylene by ACC oxidase (ACO) (Yang and Hoffman, 1984; Kende, 1993; Wang *et al*., 2002). *ACS* and *ACO* genes, which belong to multigene families, have been cloned and characterized from various different plant species. Ethylene biosynthesis is mainly regulated through the expression of *ACS* genes, but in some cases, the regulation can also be through the expression of *ACO* genes (Vriezen *et al*., 1999; Wagstaff *et al*., 2005; Fernández-Otero *et al*., 2006). The expression of *ACS* genes is regulated by many factors, including hormones, pollination, senescence, LiCl, and cycloheximide (Kende, 1993; Liang *et al*., 1996). Recent reports show that regulation of ACS activity also occurs at the post-transcriptional level (Wang *et al*., 2004; Liu and Zhang, 2004).

In *Arabidopsis*, the ethylene signalling pathway has been well characterized. Several major components in this

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pathway have been identified. Briefly, ethylene is perceived by a family of receptors that are similar to the bacterial two-component system. These receptors then activate CTR1, a Raf-like protein kinase, which acts as a negative regulator of ethylene responses. CTR1 then passes the signal to EIN2, an integral membrane protein, through a series of MAPK cascades, and then to EIN3/EILs, transcriptional factors that trigger the expression of downstream target genes such as *ERF1* (reviewed in Chen *et al*., 2005).

It has been reported that ethylene receptors are regulated at both the transcriptional and post-transcriptional levels while CTR is regulated mainly at the post-transcriptional level through association or dissociation with ethylene receptor proteins in the endoplasmic reticulum (Chen *et al*., 2002, 2005; Gao *et al*., 2003). EIN3 is highly regulated at the post-transcriptional level (Chen *et al*., 2005; Yanagisawa *et al*., 2003). Therefore, ethylene receptors are considered as a key component in ethylene signalling at the transcriptional level.

In ornamental plants, previous reports indicate that ethylene influences flower opening (Reid *et al*., 1989; Yamamoto *et al*., 1994), pollination (O'Neill *et al*., 1993; Bui and O'Neill, 1998; Dervinis *et al*., 2000), petal senescence (Shibuya *et al*., 2000; Nukui *et al*., 2004) and abscission (Kuroda *et al*., 2003, 2004). The induction of petal senescence or abscission by ethylene or pollination is associated with transcriptional regulation of the *ACS* and *ACO* genes (Bui and O'Neill, 1998; Jones, 2003; Fernández-Otero *et al*., 2006) and ethylene receptor genes (Shibuya *et al*., 2002; Kuroda *et al*., 2003, 2004). This induction is also accompanied with an increase of the *CTR* genes in some ornamental plant species (Müller *et al*., 2002; Kuroda *et al*., 2004).

Regarding the effects of ethylene on ethylene production and the expression of ethylene biosynthetic and receptor genes, several reports demonstrate that ethylene influences ethylene biosynthesis in gynoecia firstly, and then promotes petal senescence or abscission in carnation (ten Have and Woltering, 1997; Jones and Woodson, 1999; Shibuya *et al*., 2000) and orchid (O'Neill *et al*., 1993; Bui and O'Neill, 1998). It has been reported that ethylene does not affect the expression of ethylene receptor genes in gynoecia of carnation (Shibuya *et al*., 2002), geranium (Dervinis *et al*., 2000), and *Delphinium* (Tanase and Ichimura, 2006), although it does induce the flower senescence process. Therefore, more work is needed to understand the response of different floral tissues to ethylene in terms of ethylene biosynthesis and signalling in relation to ethylene-enhanced flower opening.

Flower opening in roses is sensitive to ethylene, although the degree of this sensitivity varies in different cultivars (Reid *et al*., 1989; Yamamoto *et al*., 1994; Müller *et al*., 2001; Cai *et al*., 2002; Ma *et al*., 2005; Tan *et al*., 2006). In miniature potted roses, this difference could be due to the different expression levels of receptor genes rather than ethylene biosynthetic genes (Müller *et al*., 2000*a*, *b*). Our previous work indicates that, in cut rose cv. Samantha, flower opening is regulated by ethylene, mainly through expression of two ethylene receptor and two *CTR* genes in petals but not through ethylene biosynthetic genes (Ma *et al*., 2006).

In the present work, to gain a better understanding of the ethylene response in various floral tissues and the roles of different floral tissues in ethylene-enhanced flower opening, ethylene production and the expression of five ethylene biosynthetic genes and five receptor genes in five different floral tissues of cut rose cv. Samantha upon ethylene or 1-MCP treatment were investigated. The results indicated that gynoecia may act as a key sensor to ethylene in ethylene-enhanced flower opening in roses.

Materials and methods

Plant materials

Cut roses (*Rosa hybrida* cv. Samantha) were harvested at stage 2 (completely-opened bud) from a local commercial greenhouse (Beijing, China). The flowers were immediately put in tap water after harvest and then transported to the laboratory within 1 h. After being cut to 25 cm under water, the flowers were placed in deionized water (DW) for further processing.

Treatment of flowers with ethylene and 1-MCP

Based on our previous work (Ma *et al*., 2006), 10 ppm ethylene and 2 ppm 1-MCP were used for the respective treatments. The flowers were sealed in a 64 l chamber with ethylene, 1-MCP, or regular air as the control, at 25 °C for 24 h. One mol l^{-1} NaOH was put into the chamber to prevent the accumulation of CO_2. After treatments, the flowers were placed in a vase with DW under the following controlled conditions: 23–25 °C room temperature, 30–40% relative humidity (RH), and a 12/12 h light/dark photoperiod with an illumination of ~40 μmol m^{-2} s^{-1}.

Measurements of ethylene production

The whole flower was separated into five distinct parts: sepals, petals, stamens, gynoecia, and a receptacle. To measure the ethylene production, petals of each individual flower were collected and placed in a 200 ml airtight container; the other four tissues were placed separately in a 25 ml container. Our results indicated that sepals, petals, stamens, and receptacles did not produce wound ethylene in the first 1.5 h of incubation and gynoecia did not produce wound ethylene in the first 50 min (data not shown). Thus, to avoid the contamination of wound-induced ethylene, the containers were capped and incubated at 25 °C for 1 h for sepals, petals, stamens, and receptacles, and 40 min for gynoecia. Then 2 ml sample of head space gas was withdrawn using a gas-tight hypodermic syringe, and injected into a gas chromatograph (GC 17A, Shimadzu, Kyoto, Japan) for ethylene concentration measurement. The gas chromatograph was equipped with a flame ionization detector and an activated alumina column. Ten flowers were used for independent measurements and the average values are presented.

RNA extraction in floral tissues

Total RNA from sepals, petals, and receptacles was extracted using the hot borate method as described in Wan and Wilkins (1994) and

total RNA from stamens and gynoecia was extracted using the hot phenol method. The extraction buffers in both methods were preheated to 86 °C in a water bath before use. Briefly, for the hot borate method, the extraction buffer contained 200 mM sodium tetraborate decahydrate, 30 mM EGTA, 1% deoxycholic acid sodium salt, 10 mM DTT, 2% PVP 40, 1% NP-40, and the RNA was precipitated by 2 M LiCl; for the hot phenol method, the extraction buffer contained 0.1 M TRIS (pH 9.0), 0.1 M LiCl, 1% SDS, 10 mM EDTA, and the RNA was extracted by phenol/chloroform (1:1 v/v) for three times and then precipitated by 2 M LiCl overnight. The precipitate was rewashed twice using 2 M LiCl, and then dissolved in 1 M TRIS (pH 7.5). Finally the RNA was precipitated with ethanol at –80 °C for 2 h.

RT-PCR analysis

One microgram of total RNA was used for synthesizing cDNA using Powerscript reverse transcriptase (Clontech, CA, USA) according to the manufacturer's protocol. The primers used in the RT-PCR analysis are listed in Table 1. PCR reactions were carried out for 5 min at 94 °C, followed by 33 cycles of 30 s at 94 °C, 30 s at 57 °C, 45 s at 72 °C for *Rh-ACS1–4*, 25 cycles for *Rh-ACO1* and *Ubiquitin*, and 27 cycles for *Rh-ETR1–5*, and then followed by a supplemental incubation for 5 min at 72 °C. The PCR products were separated on 1.2% agarose gels and visualized by ethidium bromide staining. Absolute values for transcript abundance from RT-PCR were quantified using the AlphaEaseFC™ 2200 software (Alpha Innotech, USA, Version 3.2.1).

Results

Ethylene production of different floral tissues treated by ethylene and 1-MCP

Our previous work indicated that ethylene treatment caused flowers of cut rose cv. Samantha to open faster and to show anthers much earlier, as well as significantly shortening the partially open period. By contrast 1-MCP significantly prolonged the partially open period and inhibited full opening of flowers by impeding the unfurling of petals in the middle and inner layers (Ma *et al*., 2006).

To understand better the temporal and spatial ethylene-induced ethylene production in different floral tissues, the morphological changes of flowers treated by ethylene and 1-MCP, respectively, were first observed. As shown in Fig. 1, ethylene treatment significantly promoted flower opening while 1-MCP significantly impeded flower opening. Ethylene production in five different floral tissues, sepals, petals, stamens, gynoecia, and receptacles, during and after ethylene and 1-MCP treatments, was then determined. During the process of ethylene treatment, gynoecia showed the most significant enhancement in ethylene production (P <0.05) at the first sampling time point of 6 h, by almost doubling that of the control, and the production reached its peak at 18 h (Fig. 2). Ethylene production in petals was obviously enhanced after 12 h treatment of ethylene, and continued increasing at the later stages. In receptacles, ethylene production showed a substantial increase after 18 h treatment of ethylene, peaked at 24 h, and was then maintained at a constant level. No obvious change in ethylene production was detected in stamens after ethylene treatment. It is worth noting that 1-MCP treatment did not suppress ethylene production in all the five tissues. Interestingly, it was found that ethylene production in sepals was dramatically decreased by ethylene treatment and was highly elevated by 1-MCP treatment.

The above results show that gynoecia have the highest sensitivity to ethylene in the very early stage of ethylene induction.

Expression of ethylene biosynthetic genes in different floral tissues treated with ethylene and 1-MCP

The expression of the five ethylene biosynthetic genes, including *Rh-ACS1–4* and *Rh-ACO1*, was then determined in the five different floral tissues treated with ethylene or 1-MCP. In gynoecia, the expression of *Rh-ACS2* and *Rh-ACS3* was enhanced substantially at 6 h of ethylene treatment (7.3 times and 1.7 times of that in the control, respectively), maintained high levels during the treatment, and then decreased dramatically after the treatment. However, their expression was not inhibited by 1-MCP. *Rh-ACS1* and *Rh-ACS4* showed a constitutive expression pattern. The expression of *Rh-ACO1* was induced at 12 h by ethylene treatment, and was also not inhibited by 1-MCP (Fig. 3).

For petals, an obvious increase of the *Rh-ACS3* expression was observed starting from 12 h of ethylene treatment, and the expression was slightly inhibited by 1-

Table 1. *Primer sequences of* Rh-ACS1–4, Rh-ACO1*, and* Rh-ETR1–5 *used in this study*

Gene	Forward primer	Reverse primer
Rh-ACS1	5′-GCGTTACAGAGGTCCTACAAG-3′	5′-ACAAACCCGGAACCAGCCTGG-3′
Rh-ACS2	5′-CCTGCCAGAGTTCAGAAATGCTGC-3′	5′-GCAATGCTGATGAACCTTGGCTGAG-3′
Rh-ACS3	5′-GCCTTGGCTTTCCTCCCTTC-3′	5′-ACCCAACTCGTCGTACGGAT-3′
Rh-ACS4	5′-GCTAATATGTCCGAGGATACTCTGG-3′	5′-CGAGTGCACTTTTCTACGTACATTTG-3′
Rh-ACO1	5′-CTCAGAAATCAAAGATTTGGACTGGG-3′	5′-CGACATTCTGTTTCCATCAGGTTGC-3′
Rh-ETR1	5′-TGTGCCATTTAGCCTTCCTGTA-3′	5′-CCTGATCTGCAACAACATCAAC-3′
Rh-ETR2	5′-CGCTATGCTTTGATGGTGTT-3′	5′-GCAGCCTACTCAGAAGGTTTT-3′
Rh-ETR3	5′-GCTCATCACTCTCATTCCTTTGC-3′	5′-GCATTGGCATCCGTATTGCAGC-3′
Rh-ETR4	5′-ACACCCTAACCCAGGTATCGTCG-3′	5′-ATGGATTGCCATCTCAGCTTCTC-3′
Rh-ETR5	5′-ATGGCTACTGCCAAGGTTTTCA-3′	5′-CAGGAATGTGTTTTCCAGCAAT-3′

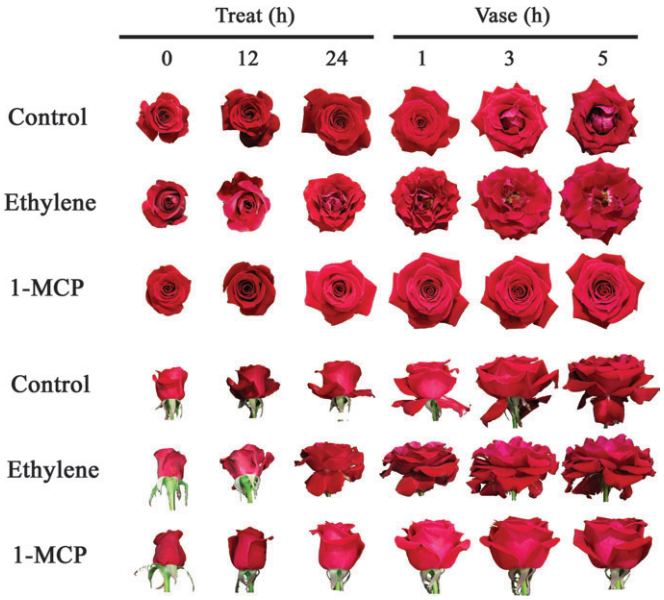

Fig. 1. Effects of ethylene and 1-MCP on flower opening of cut roses cv. Samantha. Flowers at stage 2 were treated with 10 ppm ethylene or 2 ppm 1-MCP for 24 h and then vased in deionized water (DW) for another 5 d with DW refreshed everyday. Treat: duration of ethylene or 1-MCP treatment; Vase: vase period.

MCP treatment. The expression of *Rh-ACS1* and *Rh-ACS2* stayed at a low level, and was not affected by ethylene or 1-MCP treatment. On the contrary, *Rh-ACS4* was expressed constitutively at a high level. The expression of *Rh-ACO1* was substantially increased by ethylene treatment and decreased after the treatment, and 1-MCP had very little effect on its expression (Fig. 3).

In receptacles and stamens, the expression of the five genes was not affected by ethylene or 1-MCP treatment, except that the expression of *Rh-ACS2* in receptacles was enhanced slightly by ethylene at 18 h. In sepals, however, the expression of *Rh-ACS1*, *Rh-ACS2*, and *Rh-ACO1* was greatly inhibited by ethylene treatment, and was dramatically enhanced around 18 h by 1-MCP treatment (Fig. 3).

The above results indicate that induction of the expression of certain *Rh-ACS* genes in floral tissues is consistent with the induction of ethylene production (Figs 2, 3), and gynoecia, which showed the quickest response to ethylene treatment, do not exhibit positive feedback in ethylene biosynthesis. Ethylene-induced *Rh-ACS* gene expression showed tissue-specificity; *Rh-ACS2* is mainly induced in gynoecia and receptacles, and *Rh-ACS3* is induced mainly in petals, while *Rh-ACS1* and *Rh-ACS4* are not induced by ethylene in all the tissues tested.

Expression of ethylene receptor genes in different floral tissues treated by ethylene and 1-MCP

The expression of the five ethylene receptor genes was also determined in the five floral tissues treated with ethylene or 1-MCP.

In gynoecia, the expression of *Rh-ETR1* and *Rh-ETR3* increased 2.4 and 4.7 times, respectively, at 6 h of ethylene treatment. The induction of *Rh-ETR3* expression was substantially suppressed by 1-MCP treatment, while that of *Rh-ETR1* was not reduced by 1-MCP treatment (Fig. 4).

In petals, *Rh-ETR3* expression increased significantly at 12 h of ethylene treatment, and peaked at 24 h. The expression was almost completely inhibited by 1-MCP treatment. Among the other four receptor genes, only *Rh-ETR1* expression showed a slight induction by ethylene treatment (Fig. 4).

In receptacles, *Rh-ETR3* expression was enhanced by ethylene at 18 h, and the expression was inhibited by 1-MCP during and after the treatments. In sepals and stamens, however, any effects of ethylene on the expression of the five *ETR* genes were not observed (Fig. 4).

The above results indicate that induction of *Rh-ETR* gene expression by ethylene initially occurred in gynoecia, and then in petals and receptacles. Among the five *Rh-ETR* genes, the induction of *Rh-ETR3* expression was the most obvious, and exhibited a positive feedback response.

Discussion

Flower opening is a natural and programmed plant process for propagating their offspring. During this process, petals unfold for flower pollination, and then drop afterwards. It has been documented that for ethylene-sensitive plants, ethylene is involved in flower opening and senescence (Woltering and van Doorn, 1988; van Doorn, 2002).

In natural senescence of carnation, the onset of ethylene production was in the order of ovary, styles, and petals, and the order was consistent with the expression level of *ACS* and *ACO* genes in the corresponding tissues. When flowers were treated with ethylene, both ethylene production and biosynthetic genes were induced faster in the ovary than in petals (ten Have and Woltering, 1997). Pollination-induced *ACS* gene expression in styles was severely inhibited by norbornadiene (NBD), an ethylene action inhibitor (Jones and Woodson, 1999). Shibuya *et al*. (2000) found that, when gynoecia were removed, flower vase life was prolonged substantially and ethylene production and the expression of ethylene biosynthetic genes were completely inhibited. Ethylene production in gynoecia-removed flowers was induced by exogenous ethylene, but not by ACC, ABA or IAA (Shibuya *et al*., 2000). The application of ABA or IAA accelerated flower senescence and the induction of ethylene production in gynoecia in a long-lasting carnation cultivar, whose ethylene production in petals and gynoecia was below the limit of detection during natural senescence (Nukui *et al*., 2004).

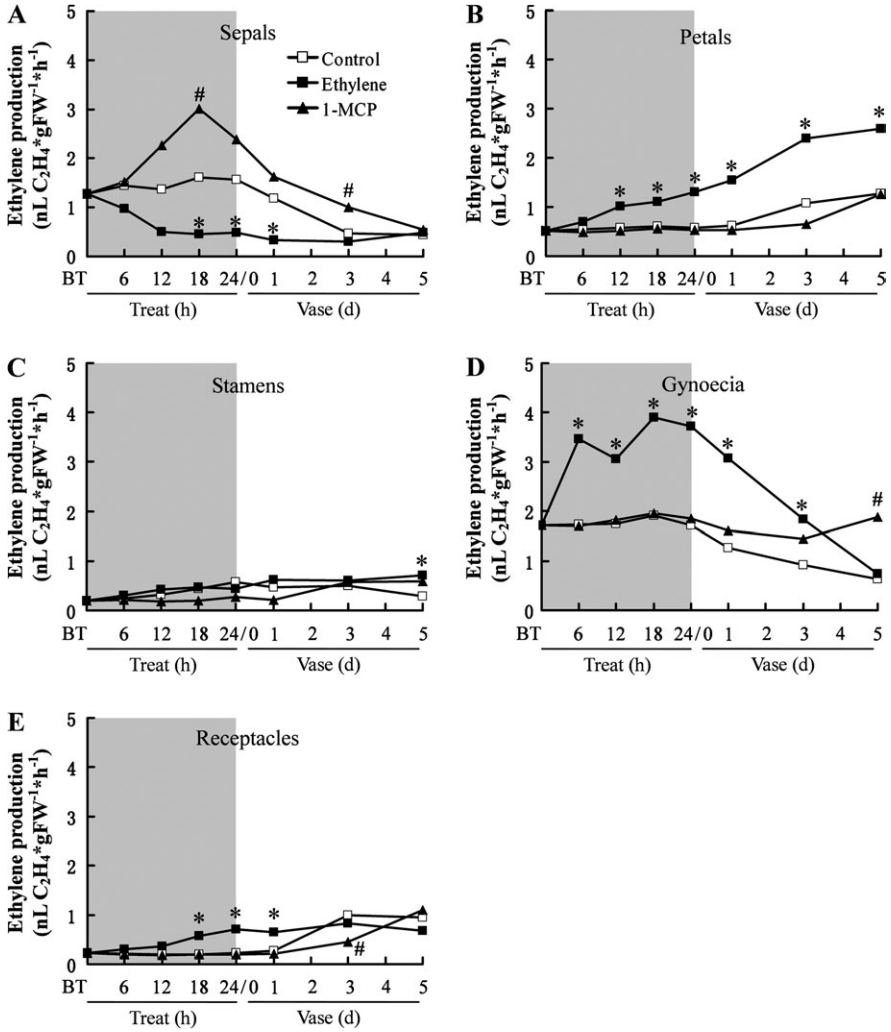

Fig. 2. Ethylene production in five floral tissues of cut rose. The flowers were treated with 10 ppm ethylene (solid squares) and 2 ppm 1-MCP (solid triangles), respectively, and in air as the control (open squares) for 24 h from BT (before the treatments) to 24 h of the treatments (also shown as 0 d, immediately after the treatments, in the *x*-axes) (grey parts), then put into deionized water in a vase for another 5 d (white parts). The * and # symbols above each data point indicated significant difference (Student's *t* test, $P < 0.05$) of ethylene production upon ethylene and 1-MCP treatment, respectively. Treat: duration of ethylene or 1-MCP treatment; Vase: vase period. Each bar represents the standard error, $n=10$.

In orchid flowers, after pollination, ethylene production increased first in gynoecia, and then in petals. The expression of one *ACS* gene (named *AS*) also increased initially in gynoecia, while its expression was undetectable in petals. The expression of *AS* in gynoecia could be completely blocked by NBD (O'Neill *et al*., 1993).

It is well known that both carnation and orchid exhibit the typical ethylene autocatalytic feedback of ethylene biosynthesis in petals. The observation of carnation and orchid above lead to the conclusion that induction of *ACS* gene expression occurred in gynoecia first, and then in petals, and the induction in gynoecia is also regulated in a positive feedback manner.

In roses, it has been proved that ethylene is involved in regulating flower opening (Reid *et al*., 1989). During the senescence process of miniature potted roses, the expression of the *ACS* gene increased in a short-lasting cultivar, but remained at a constant and low level in a long-lasting one, while the expression of the *ACO* gene increased in the final stage of flower development in both cultivars (Müller *et al*., 2000*a*).

Our previous work showed that ethylene treatment induced *Rh-ACS3* expression in rose petals (Ma *et al*., 2005), while 1-MCP did not suppress ethylene production or *Rh-ACS3* expression in petals but impeded full flower opening effectively (Ma *et al*., 2006). Therefore, there should be no positive feedback in ethylene-induced ethylene biosynthesis in rose petals. In the present work, to investigate how ethylene affects the expression of ethylene biosynthetic genes in five different floral tissues, ethylene production and expression of ethylene biosynthetic genes were determined in these tissues upon

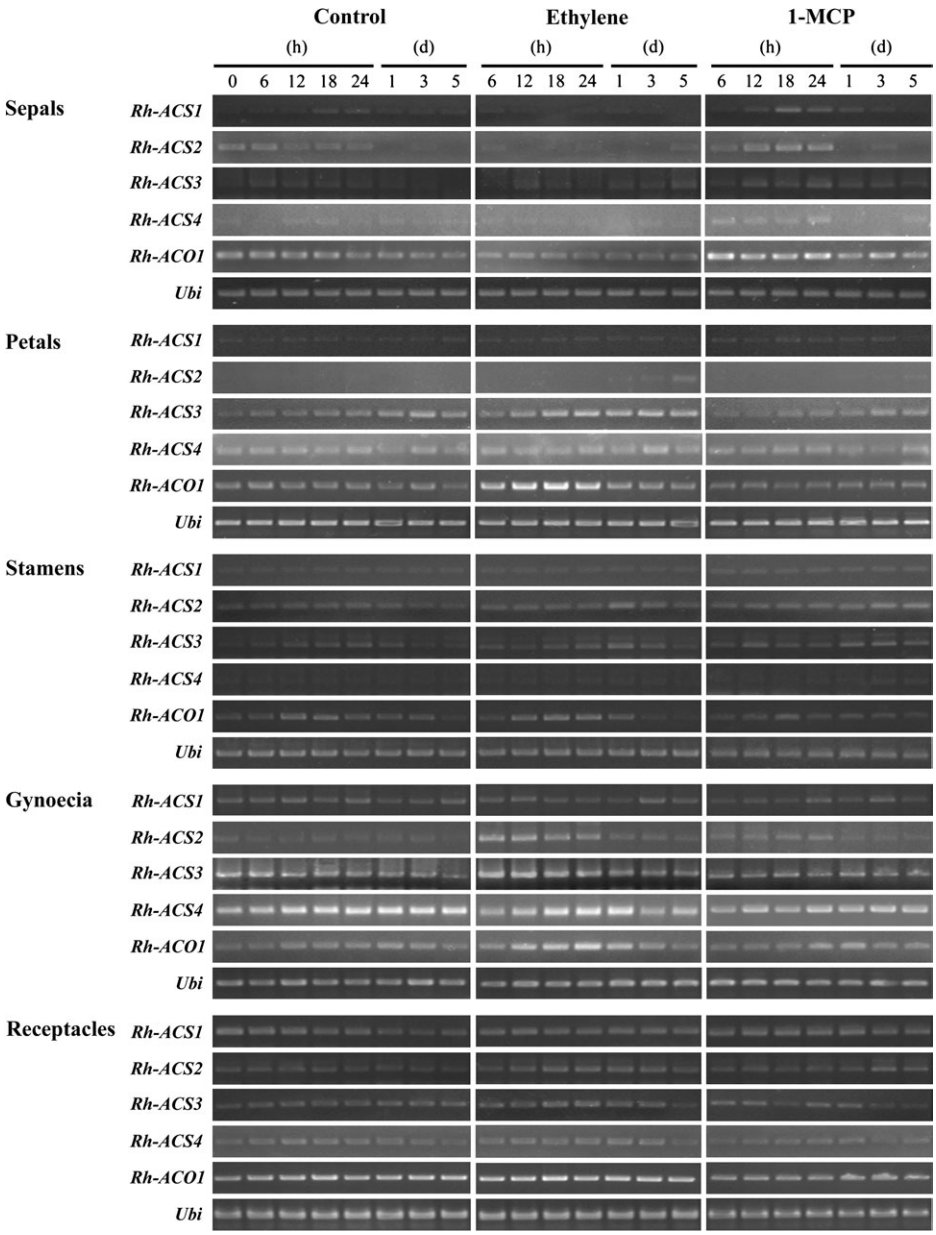

Fig. 3. Expression of *Rh-ACS1–4* and *Rh-ACO1* genes in various tissues of cut rose flowers determined by RT-PCR. Flowers were treated with 10 ppm ethylene and 2 ppm 1-MCP respectively, and in air as the control, for 24 h. 0–24 (h), treatment time of ethylene or 1-MCP; 1–5 (d), vase time. *Ubiquitin* gene was used as an internal control.

ethylene or 1-MCP treatment. It was found that ethylene enhanced both ethylene production and the expression of *Rh-ACS2* or *Rh-ACS3* in gynoecia, petals, and receptacles, with gynoecia showing the earliest ethylene enhancement. However, 1-MCP did not suppress ethylene production and the expression of the ethylene biosynthetic genes in these three tissues (see Figs 2, 3). In stamens, no obvious changes were found in ethylene biosynthesis by either ethylene or 1-MCP treatment. The present results, together with our previous observations, suggest that gynoecia are the most sensitive tissue to ethylene treatment, and none of the five floral tissues exhibits positive feedback regulation of ethylene biosynthesis.

Our previous work showed that, in petals, there were different induction features in three *ACS* genes of roses, and *Rh-ACS2* was induced by senescence (Ma *et al*., 2005). In the present work, tissue-specificity of different *ACS* genes was also observed (Fig. 3) and *Rh-ACS2* was quickly induced by ethylene in gynoecia. Similar observations were reported in carnation, where *CARAS1* (also named as *DC-ACS2*) showed a quicker and stronger response to ethylene treatment in gynoecia than in petals

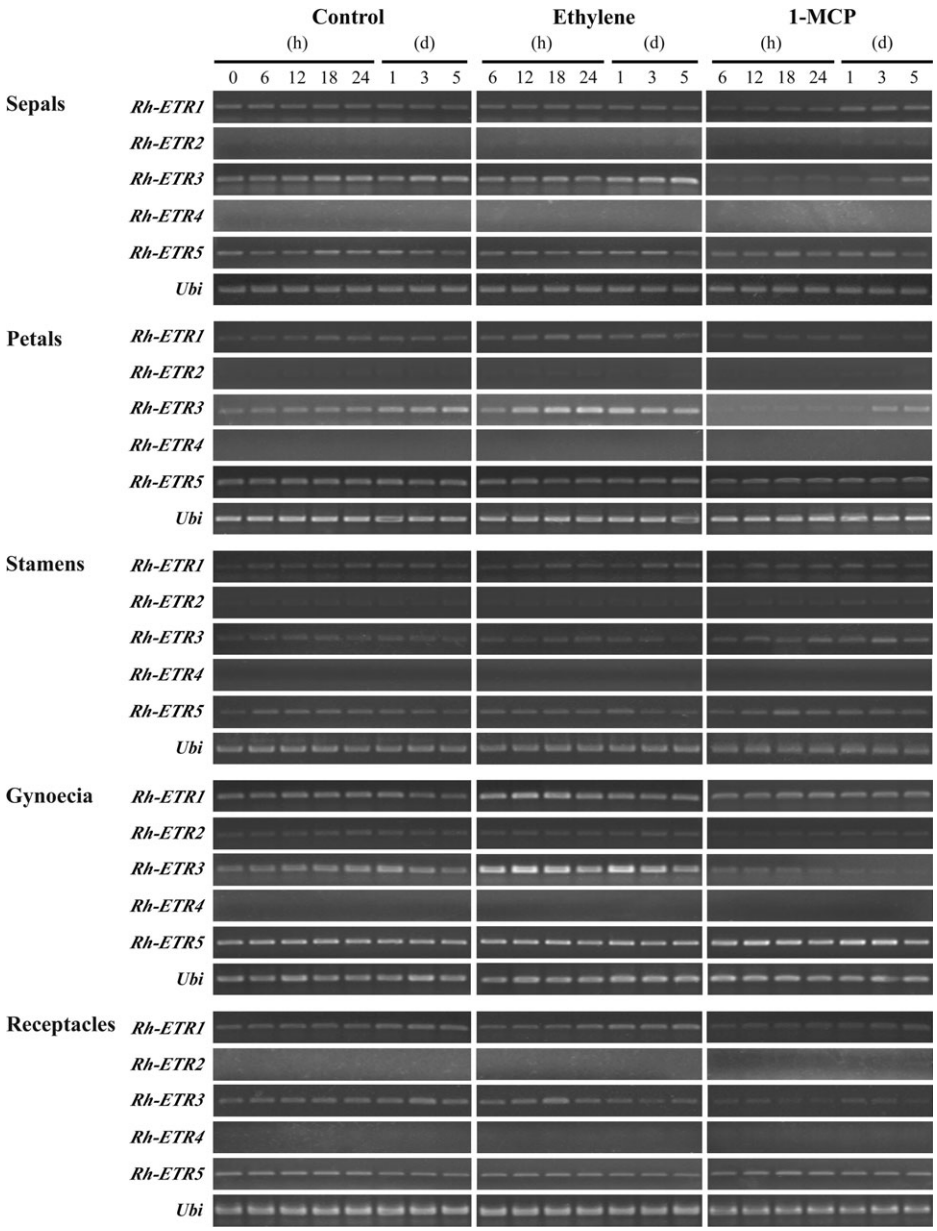

Fig. 4. Expression of *Rh-ETR1–5* in various tissues of cut rose flowers determined by RT-PCR. Flowers were treated with 10 ppm ethylene and 2 ppm 1-MCP respectively, and in air as control, for 24 h. 0–24 (h), treatment time of ethylene or 1-MCP; 1–5 (d), vase time. *Ubiquitin* gene was used as an internal control.

(ten Have and Woltering, 1997). These observations suggest that *Rh-ACS2*, a senescence-associated gene in rose petals, may play an important role in the induction of ethylene biosynthesis in gynoecia, and therefore may be involved in promoting the flower opening process.

Müller *et al*., (2000*a*, *b*) compared the expression of ethylene receptors in petals of two cultivars of miniature potted roses during flower development. The expression of *RhETR1* was higher in a short-lasting cultivar than in a long-lasting one, and *RhETR3* expression was increased in senescing flowers of the short-lasting cultivar, while it remained at a low level in the long-lasting one. *RhETR2* was expressed at a constitutive level throughout flower development. Ethylene induced the expression of all the three receptors. In chrysanthemum, the expression of *DG-ERS1* in petals was higher in the ethylene-sensitive cultivar than in the ethylene-insensitive cultivar during senescence or after ethylene treatment (Narumi *et al*., 2005). These observations suggest that the variation in flower longevity or ethylene sensitivity can be attributed to the differential expression of ethylene receptor genes in petals.

Several reports described the expression of ethylene receptor genes in different floral tissues. During flower

senescence in carnation, *DC-ETR1* and *DC-ERS1* were expressed constitutively or at undetectable levels in all tissues; and the expression of *DC-ERS2* was not affected by ethylene treatment, although after ethylene treatment, its expression increased slightly in ovaries, decreased in petals, and remained unchanged in styles (Shibuya *et al*., 2002). In geranium, self-pollination and ethylene treatment induced petal abscission, while they did not affect the expression of *PhETR1* and *PhETR2* in pistils and receptacles (Dervinis *et al*., 2000). In *Delphinium*, the expression of receptor genes was not affected by ethylene in gynoecia or receptacles, although they were induced developmentally in abscised florets and sepals (Kuroda *et al*., 2003, 2004; Tanase and Ichimura, 2006). These results indicate that the influence of ethylene on flower senescence in these plants may not proceed through regulation of ethylene receptor gene expression in gynoecia.

Previous work indicates that, in rose petals, ethylene regulates flower opening mainly through the expression of two ethylene receptor genes (*Rh-ETR1* and *Rh-ETR3*) and two *CTR* (*Rh-CTR1* and *Rh-CTR2*) genes (Ma *et al*., 2006). In the current work, it was found that, among the five floral tissues studied, ethylene-induced expression of *Rh-ETR* genes occurred first in gynoecia, and only the expression of the *Rh-ETR3* gene was regulated in a positive feedback manner by ethylene and 1-MCP (Fig. 4). These results suggest that transcriptional regulation of the *Rh-ETR3* gene in gynoecia may play an important role in ethylene-enhanced flower opening. These results differ from the findings in carnation, geranium, and *Delphinium* mentioned above. Further investigation is required to understand the difference.

In the present work, it was found that, in rose sepals, ethylene production was significantly inhibited by ethylene treatment, while promoted greatly by 1-MCP (Fig. 2). The inhibition and promotion of ethylene production by ethylene and 1-MCP, respectively, were consistent with changes in ethylene biosynthetic gene expression levels (Fig. 3). The phenomenon of ethylene auto-inhibition was also found in tobacco leaf discs (Philosoph-Hadas *et al*., 1985), carnation leaves (Henskens *et al*., 1994), and *Pelargonium* cuttings (mainly comprised of leaves and stems) (Mutui *et al*., 2007). In addition, it has been reported that in the calyx of detached young persimmon fruit, 1-MCP promoted ethylene production, and the promotion was consistent with that of *DK-ACS2* expression (Nakano *et al*., 2003). All of the tissues mentioned above, in which ethylene production was inhibited by ethylene and promoted by 1-MCP, belong to vegetative tissues, indicating that a common regulatory mechanism of ethylene production could be shared by these different vegetative tissues.

Ganelevin and Zieslin (2002) found that removing sepals from rose flowers at the early opening stage produced flowers with a 'star-shape', and this phenomenon was similar to that of ethylene-treated flowers (Reid *et al*., 1989). These findings suggest that sepals may play a role in ethylene-enhanced flower opening through influencing flower sensitivity to ethylene.

Acknowledgements

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