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Study of the relationship between senescence and relative expression of an ethylene receptor gene in *Rosa hybrida* cv. Cool Water in response to exogenous ethylene

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ABSTRACT

Although roses are not classified as highly sensitive to ethylene, their response to ethylene varies .In this study, we examined the effect of exogenous ethylene on apparent properties of cut Rose (Rosa hybrida) cv. Cool Water with low vase life through quantifying the ethylene receptor gene, RhETR3. The cut flowers were treated with 10 ppm ethylene concentration and the expression of ethylene receptor gene was measured before ethylene treatment, 24 h, 48 h and 72 h after ethylene treatment via real-time RT-PCR. We found that the level of relative expression ethylene receptor gene, RhETR3 had significant increase with ethylene treatment during senescence and ethylene caused accelerated of senescence symptoms such as petal fading and bent neck. Moreover, the relative expression of RhETR3 remained in low level in controls because of its negative regulatory function.

Keywords: *Rosa hybrid*, ethylene**,** expression**,** *RhETR3***,** real-time RT-PCR

INTRODUCTION

Roses are the most important cut flowers in the flower industry [13]. Apparent quality and vase life of roses are influenced by many factors. One of these factors is the presence of ethylene in the atmosphere of cut flowers during transport and handling, which affect the quality of cut roses [24]. Ethylene is involved in many aspects of plant growth and development such as germination, flower and leaf senescence and abscission. Also, ethylene is the primary promoter in senescence and abscission of floral organs in a wide range of flowering plants [23].

Roses are classified as sensitive to ethylene [22], but the role of external ethylene in flowers from accelerated or inhibited of opening flower or senescence depends on rose variety [14,17]. To perceive the ethylene by plant tissues, this gaseous molecule binds to receptor proteins in plant cells and activates the transcription and translation of downstream genes [7, 23]. The activation of genes is resulted in a set of reactions that eventually leads to senescence and the death of cell and organs [23]. Study on ethylene receptor genes in *Rosa hybrida* has been understood through the studies on the gene conferring *ETR1* in Arabidopsis [2, 5]. Some ethylene receptor genes have been identified in *Rosa hybrida* including *RhETR1*, *RhETR2*, *RhETR3*, *RhETR4* and *RhETR5* [16, 19]. Based on the molecular studies for ethylene resistance, ethylene receptor genes in roses such as *RhETR1*, *RhETR3* and *RhETR5* caused differences in display quality and vase life. For example, in rose cultivars such as Bronze and Vanilla, which have different vase life, the expressions of these receptors are different [15, 19]. Therefore, we hypothesized that the expression of *RhETR3* is more affected by ethylene in rose flowers. The aim of this study was to understand the

changes in the expression of *RhETR3* during senescence in response to exogenous ethylene and to understand the relationship between onset of senescence and relative expression of ethylene receptor gene, *RhETR3*.

MATERIALS AND METHODS

Plant materials

Rosa hybrida cultivars 'Cool Water' were harvested at stage 2 (completely open bud) [11] from a local commercial green house (Bijar, Iran). We chose this cultivar because of its short vase life (approximately 6 day). Cut flowers were immediately put in tap water after harvest and transported to the laboratory within 1 h. cut flowers were placed in deioniezed water (DW) for 1 h to be dehydrated before treatment with ethylene. Then they were cut to 25 cm and were placed in DW. During the experiment, flowers were kept in DW and at controlled conditions ,23–25 ˚C, with 30–40% relative humidity, and a 12 h light:12 h dark photoperiod provided by fluorescent lights (80 $\mu molm^{-2}\ s^{-1}$) [19].

Exogenous ethylene treatment

Based on previous work by Ma *et al*., (2006) [12], 10 ppm ethylene was used in order to evaluate its effects on expression of ethylene receptor gene (*RhETR3*). The flowers were sealed in plastic chambers and aliquot of pure ethylene gas were injected by syringe in to the chambers to achieve treatment concentration. Control flowers were placed in the chambers under the same conditions without ethylene injection. After 24 h exposure to ethylene, chambers were opened and ventilation occurred. Petal samples were collected before and in 24 h, 48 h and 72 h after being treated with ethylene and were frozen immediately in liquid nitrogen and then stored at -80˚C until extraction of RNA were performed.

RNA extraction

Total RNA from petals was extracted using CTAB (cetyl trimethylammonium bromide)-based methods described by Chang [3] with little modification. Briefly, 0.1g of grounded tissues was homogenized after the addition of ß-marcaptoethanol to 1 ml of preheated extraction buffer (300mMTris-HCL, pH=8, 25mMethylenediaminetetra acetic acid (EDTA), pH=8.0, 2MNaCl, 2% (w/v)soluble PVP(Sigma, 40,000 MW) and 2%(w/v) CTAB (Sigma)).The nucleic acids were precipitated after addition of chloroform: isoamyl alcohol (24:1) with 3M sodium acetate (NaOAc) at pH=5.2. Addition of 8MLiCl over night at 4 ˚C resulted in selective precipitation of total RNA. Then, the pellets of RNA were centrifuged and washed with 70% ethanol and the extracted RNA was stored at -80˚Cfor further studies.

Quantification and Quality Control

The extracted nucleic acids were quantified at wavelengths of 230 nm, 260 nm, and 280 nm (A260/A230 and A260/A280 ratios) with Nano Drop instrument. The integrity of total RNA was verified by running samples on 1.2% agarose gel containing ethidium bromide (EtBr)[1].

cDNA Synthesis and qRT-PCR

To synthesize cDNA, the 5 µg of extracted total RNA treated with DNase I (Fermentas) were used as a template using Oligo $(dT)_{18}$ primer (1 µg/µl, vivantis) for 5 min at70˚C.After being cold, the reaction mixture was incubated with M-MuLV Reverse Transcriptase (100 u/µl, vivantis) for 60 min at 42˚C. Heating the mixture at 70˚C for 10 min resulted in inactivation enzymes.

Table 1: Gene-specific primer pairs used for real-time RT-PCR

gene	Accession number	Primer pairs	Sequences (5′-3′)
Rh ETR3	AF154119	Forward primer	GGGCCAGATTCAATACTCGT
		Reverse primer	ATCTCAAGTTCCTGGCTGCT
Rhß-actin	AB239794	Forward primer	CCACAGCTGAGCGAGAAATA
		Reverse primer	GTACTTCTGGGCAACGGAAT

Real Time RT-PCR assays were performed using the Rotor Gene 3000 real time thermal cycler (Corbett Life Science Co.) using RealQ-PCR2×Master Mix kit (Amplicon, Denmark) according to the recommendations of the manufacturer. Reaction mixtures (25µl) contained 12.5µl 2× SYBR-Green reaction mixed with 0.8µl each of gene-specific forward and reverse primer designed with Dnastar and Oligo programs (Table 1), 3µl cDNA and 7.9µl DEPC H_2O. The thermal profile used consisted of 15 min at 95˚C, then followed by 40 cycles of 95 ˚C for 25s, 57˚C for1min and 72˚C for 30 s and completed with a melting curve analysis program. An endogenous *Rhß-actin* was used as an internal standard. Relative expression levels were calculated using the delta threshold Cycle (Ct) methods and using $2^{-\Delta\Delta CT}$ [10].

Statistical analyses and bioinformatics
Randomized completely blocks designs with two independent biological replications and two technical replications were used. Analysis of variance of the data from $2^{-\Delta\Delta CT}$ method was performed using MSTAT-C software and means were compared by the least significant difference (LSD) test at the 0.05 and 0.01 probability levels.

RESULTS AND DISCUSSION

Statistical analysis of data from quantitative Real-Time PCR showed that relative expression of *RhETR3*, significantly increased with ethylene treatment ($\alpha < 0.01$) .The relative expression of ethylene receptor gene, *RhETR3*, during senescence was also significant ($\alpha < 0.05$) compared to controls (data not shown). Fig. 1 showed that in control flowers the relative expression of gene, *RhETR3* decreased with time and then remained at a low level. Our results were inconsistence with the findings of Hua and Meyerowitz [6] who used Arabidopsis, Tieman *et al.* [20] who used tomatoes and Shibuya *et al*. [18] who used carnation. They found that ethylene receptors function as negative regulators of ethylene responses in the signal transduction pathways. This means that there is a negative correlation between receptor level and sensitivity to ethylene. Therefore, more ethylene is needed to deactivate levels of receptors [4].

Relative expression of *RhETR3* increased significantly ($\alpha<0.05$) during senescence and 72 h after treatment with ethylene. In Arabidopsis relative expression of *ER2*, *ERS1* and *ERS2* genes increased with exogenous ethylene [7]. Also in tomatoes, a significant change in expression of receptors in response to ethylene was observed and desired genes expression increased [8]. In miniature potted roses, ethylene receptors expressed significantly by exogenous ethylene [16] that was in consistence with the results of this research. Study of the apparent features of Cool Water showed that in untreated flowers, onset of senescence coincide with symptoms such as fading and bent neck, which accurse 6 days after harvest. In flowers treated with ethylene, senescence symptoms were observed in third day. Investigation of relative expression of ethylene receptor gene and onset of senescence symptoms represents the correlation between them. Along with the onset of senescence in treated flowers, relative expression of ethylene receptor gene, *RhETR3*, increased in 72 h after ethylene treatment (Fig. 1). This was in consistence with the theory that ethylene would lead to appearance of senescence symptoms in the varieties which are sensitive to ethylene [9, 18, 21].

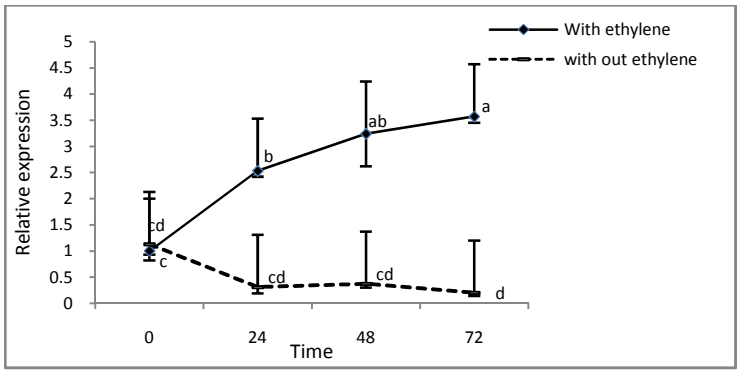

Figure 1: Relative expression level of ethylene receptor gene, *RhETR3*, before and during 24h, 48h and 72 h after ethylene treatment and in untreated flowers (controls). Bars are standard error of the means.

CONCLUSION

In conclusion, in *Rosa hybrida* cv. 'Cool Water' with short vase life, low level of relative expression of *RhTER3* in controls indicated more sensitively to ethylene because of the negative regulatory function of ethylene receptors, *ETRs*. The level of relative expression ethylene receptor gene, *RhTER3* gradually increased in cv. Cool Water due to the effect of exogenous ethylene on accelerated of senescence symptoms such as petal fading and bent neck. Therefore low level of ethylene receptor in 'Cool Water' control flower indicates that this cultivar is sensitive to ethylene, but to make a decision for commercial purposes based on the results of this study, we need to evaluate the ethylene production.

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