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Effects of 1-MCP and ethylene on postharvest quality and expression of senescence-associated genes in cut rose cv. Sparkle

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ABSTRACT

The aim of this study was to investigate the effects of 1-methylcyclopropene (1-MCP) and ethylene on postharvest quality of cut rose 'Sparkle' and expression of Laccase and Aquaporins genes. Cut flowers were pre-treated with 0, 0.5 or 1 $\mu L L^{-1}$ of 1-MCP concentrations for 12 h and subsequently exposed to 8 $\mu L L^{-1}$ of exogenous ethylene for 6 h. Control treatment was received neither 1-MCP nor ethylene. Molecular study was carried out on petals of flowers pre-treated with 0 or 1 $\mu L L^{-1}$ 1-MCP exposed to 8 $\mu L L^{-1}$ ethylene. The results showed that 1-MCP treatment did not have significant effects on flower diameter, relative fresh weight, solution uptake, and water loss, while it reduced ethylene production and chlorophyll degradation, and increased postharvest longevity. Control and ethylene treated cut stems revealed 2 days differences in postharvest longevity of 'Sparkle'. 1-MCP suppressed the expression of all investigated genes except *RhPIP1*. The expression of *RhLAC* and *RhTIP1* was induced by ethylene. Physiological and molecular data showed that this cultivar is sensitive to exogenous ethylene, while 1-MCP treatment inhibited ethylene's detrimental effects. Therefore, applying 1-MCP or other ethylene blocking agents are recommended as a postharvest treatment for extending the postharvest quality of cut rose 'Sparkle'.

1. Introduction

Ethylene, as a simple hydrocarbon gaseous hormone, affects many physiological processes in plants from seed germination and root formation to fruit ripening and organ abscission (Li et al., 2001). Ethylene is received through binding to a family of membrane-localized proteins called ethylene receptors such as ETR1, ETR2, ERS1, ERS2, and EIN4 (Bleecker and Kende, 2000). After perception of ethylene, activation of signal transduction cascade affects the expression patterns of ethylene-dependent genes (Davies, 2004; Mcgrath and Ecker, 1998; Serek et al., 2006).

Ethylene plays important role in the postharvest longevity and display quality of ornamental plants, including roses. Research showed that exogenous ethylene induced flower organs abscission in miniature potted *Rosa hybrida* cvs. 'Charming', 'Bronze', 'Lavender', 'Vanilla', and their F_1 breeding lines (Müller et al., 1998, 2000; Ahmadi et al., 2009), 'Belle Sunblaze' (Tjosvold et al., 1995), 'Victory Parade' (Serek et al., 1994), and cut rose cv. 'Marlyse' (Ait-Oubahou et al., 2003). Ethylene negatively affected the display quality of *Rosa bourboniana* var. 'Gruss an Teplitz' too (Sane et al., 2007; Tripathi et al., 2009). Treatment of rose plants or cut flowers with ethylene action inhibitors such as 1-methylcyclopropene (1-MCP) delayed senescence and abscission processes in *R. hybrida* 'Bronze', 'Vanilla' (Müller et al., 2000), resulted in extending of postharvest characteristics. Application of 1-MCP improved display life of various cultivars showed high and low sensitivity to exogenous ethylene (Da Rocha Batista et al., 2008). Hunter et al. (2004) showed pre-treatment of *Narcissus pseudonarcissus* L. 'Dutch Master' with 500 $nL L^{-1}$ 1-MCP resulted in delayed flowers senescence and transcript accumulation caused by exogenous ethylene. 1-MCP is a strong ethylene action inhibitor with high ability to bind permanently to the receptors and suppresses the ethylene response pathway in plants. 1-MCP may have a wide range of effects on respiration and ethylene production and consequently delaying chlorophyll degradation (Blankenship and Dole, 2003).

Aquaporin (AQP) proteins play a hub role in plant growth phenomenon acting as water channel proteins in vacuolar and plasma membranes (Ma et al., 2008). These proteins are classified into four subgroups including plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs) (Chaumont et al., 2001; Johanson and Gustavsson, 2002; Johansson et al., 2000). It was reported that ethylene suppressed the expression of *PIPs* and *TIPs*, while 1-MCP treatment induced the expression of these genes in *R. hybrida* 'Samantha' (Ma et al., 2008). The full length of Laccase (Lac) gene expressed in *R. hybrida* 'Lavender' under ethylene treatment was isolated by Ahmadi et al., 2008. Based on collected data, this gene can possibly be involved in organ abscission

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

Gene	Accession number	Primer pair	Sequence (5′–3′)
RhPIP1	EV572717	Forward primer	GCCATGATCTTTGTCCTCGT
		Reverse primer	AATGGTCCGACCCAGAAGAT
RhTIP1	BAB12727	Forward primer	CAACAAACTCACCGACAACG
		Reverse primer	TTCTCCCAGTTCCAGCTCAC
RhLAC	EU603403	Forward primer	GAACCACCCCATTGATGTTC
		Reverse primer	TGGCAGTCAGCATAAACCAA
Rhßactin	AB239794	Forward primer	TGCTCCCGCTATGTATGTTG
		Reverse primer	GGACTTCTGGGCATCTGAAA

and acceleration of senescence, especially in high-ethylene sensitive genotypes of miniature roses (Ahmadi et al., 2009). Although, southern blot analysis revealed that there are multiple copies of RhLAC gene in Rosa species (Ahmadi et al., 2008), there is no information relating to expression behavior of this gene under applying blocking agents of ethylene receptors.

The purpose of this study was to investigate the effects of ethylene and 1-MCP on postharvest quality of cut rose 'Sparkle' and on the expression of Laccase (Lac) and Aquaporins (AQP) genes. Application of 1-MCP gains a better understanding of expression pattern of Lac in downstream of ethylene signal transduction pathway.

2. Materials and methods

2.1. Plant material

R. hybrida cv. 'Sparkle' flowers were harvested from a commercial greenhouse based on commercial harvesting index (petal reflexing). Flowers were immediately brought to the laboratory, Tarbiat Modares University, Tehran, Iran and the length of stems was re-cut to adjust to 45 cm and placed in deionized water (DW).

2.2. 1-MCP and ethylene treatments

Cut rose stems were placed in 200 L glass chambers and treated with desirable concentrations of 1-MCP (0, 0.5 and 1 $\mu L L^{-1}$) for 12 h. After adding some drops of water to commercial powdered formulation SmartFresh™ (AgroFresh, USA), immediately the chambers' lids were tightly sealed according to Buanong et al. (2005). 1-MCP-treated and non treated flowers were aerated for an hour and thereafter were exposed to 8 $\mu L L^{-1}$ ethylene concentration for 6 h. Control cut flowers were placed in identical glass chamber without receiving 1-MCP or ethylene treatment. A 100 ml solution of 1 M NaOH was also placed inside the chambers to maintain low CO_2 concentrations from respiration during both treatments. The experiment was conducted at 20 ± 2 °C, 60–65% RH, and a 12/12 h light/dark photoperiod at an illumination of 15 $\mu mol\ m^{-2}\ s^{-1}$. After ethylene treatment, rose petals were immediately collected, frozen in liquid nitrogen and stored at −80 °C for RNA extraction.

2.3. Evaluation of display quality characteristics

Water uptake (WU), water loss (WL, transpiration) rates and relative fresh weight (FW) were evaluated according to Çelikel et al. (2011) during the experiment. Leaf chlorophyll content was measured using three replicates of ten leaf blades for each treatment as described by Ahmadi et al. (2009). Flower opening was measured on days 1, 3, 5, 7, 9 and 11 by digital caliper. Flower longevity was evaluated according to Possiel (2008).

2.4. Ethylene measurement

Immediately after ethylene treatment three flowers from each treatment were placed in a 1.8 L sealed glass bottle, and kept at 20 ± 2 °C for 48 h. Gas samples were taken after 24 and 48 h through headspaces via gas-tight syringe and injected into a gas chromatograph (GC Agilent 6890N) fitted with a capillary column and a flame ionization (FID) detector. The carrier gas was helium at 6.5 mL min^{-1}, injection temperature was 180 °C and column temperature was 60 °C.

2.5. Extraction of RNA and reverse transcription

Total RNA was extracted from petal samples pre-treated with 1-MCP and treated with ethylene or samples received only ethylene. Total RNA was isolated from 80 to 100 mg of ground petal samples using the Invisorb® Spin Plant RNA Mini Kit (Invitek Co.) according to the manufacturer's protocols. To evaluate RNA quality and quantity, total RNA was fractionated on 1% agarose gel, visualized by staining with GelRed™ and compared with standard DNA concentration. For removing any genomic DNA, RNA samples were treated with DNase I as follows: 0.25 U of DNase I, 2 μL of 10× DNase reaction buffer (Vivantis Co.), 20 U of RNase inhibitor (Takara co.), 4.75 μL sterilized distilled water added to each 12.5 μL of RNA samples and incubated in a thermocycler (Bio-Rad co.) at 37 °C for 30 min. To eliminate residual DNase, 0.1 mM of EDTA (CinnaGen Co.) was added to each RNA sample and incubated at 65 °C for 10 min. To avoid RNA degradation, samples were stored at −20 °C for short term or −80 °C for long term until further processing. First cDNA strands were synthesized in Laboratory of Horticultural Science Department, Tarbiat Modares University, Tehran, Iran. Adding 0.5 μg of oligo$(dT)_{18}$ primer and 4.5 μL sterilized distilled water added to desirable concentration of RNA, then the reaction tubes were incubated for 10 min at 70 °C and quenched immediately on ice for 5 min. For each reaction, 1 μL of M-MLV RT 10× buffer, 0.5 mM dNTPs, 200 U of M-MLV RT (H−) enzyme, and 20 U of RNase inhibitor (Takara Co.) were added to each tube incubated 2 h at 42 °C.

2.6. Real-time RT-PCR assays

To evaluate mRNA level expression, real-time RT-PCR assays were prepared using the Rotor Gene 3000 real-time thermal cycler (Corbett Life Science Co.) at the laboratory of Floriculture Section, Leibniz University Hannover, Germany. The PCR reaction mixture was made up to a volume of 20 μL containing 10 ng of cDNA template, 150 μM of each dNTP, 0.25 μM forward primer, 0.25 μM reverse primer (Table 1), 1 U of Taq DNA polymerase (Axon Co.), 5 mM $MgCl_2$, 10 mM Tris-HCl and 50 mM KCl. PCR Amplification was monitored via intercalation of SYBR® Green (Roche Applied Science Co.) added to the reaction mixture in each tube. After 3 min of incubation at 94 °C, the cDNA was amplified by 40 three-step cycles: 30 s at 95 °C, 1 min at 61 °C, and 2 min at 72 °C. Each