Postharvest Biology and Technology 131 (2017) 74–83

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Relationships between the longevity, water relations, ethylene sensitivity, and gene expression of cut roses

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ARTICLE INFO

Keywords:
Ethylene biosynthesis
Ethylene sensitivity
Gene expression
Postharvest
Potential vase life
Rose

ABSTRACT

Cut roses (*Rosa hybrida* L.) have been classified as ethylene-sensitive, but the nature of the ethylene sensitivity changes in these flowers has not been well characterized. In the present study, the relationships between vase life, ethylene sensitivity, and expression of ethylene biosynthetic genes were determined in 33 cultivars. Despite the same growing season and identical postharvest environments, the vase life of cultivars ranged from 5.5 to 15.5 d. Ethylene primarily accelerated petal wilting, which is the main factor shortening the longevity in long vase life cultivars. qRT-PCR analysis revealed that among five ethylene synthesis genes, *RhACO1* showed the most distinct expression patterns in response to ethylene. ACS genes were differentially expressed in response to ethylene, and only *RhACS2* and *RhACS4* are induced by ethylene and during flower senescence. Correlation analysis revealed that the major factors that terminate vase life vary depending on flower sensitivity to ethylene. The vase life of ethylene-sensitive cultivars was strongly related to *RhACO1* transcript level, which was induced by ethylene exposure. In contrast, the vase life of ethylene-insensitive cultivars was mostly reduced due to an early failure of water relations, as a consequence of the decreased hydraulic conductance of stems and water loss via transpiration. The current study revealed that postharvest treatments to improve the vase life should be differently applied based on the ethylene sensitivity of each cultivar.

1. Introduction

The potential vase life (longevity) of cut flowers depends primarily on phenotypic factors, which are determined by the genotypic characteristics of cultivars and on pre-harvest environmental conditions (Wu et al., 1991; Muller et al., 1998; Mortensen and Gislerød, 1999). The longevity of cut roses is often shortened to the early stages of maturation due to wilting, abscission, discoloration, and neck bending (bent neck) of floral organs or leaves. These manifestations of deterioration observed in cut roses result from the complex interaction of myriad physiological processes occurring in the flowers, leaves, and/or stems that comprise each floral unit (Halevy and Mayak, 1981; Zieslin, 1989; In et al., 2007).

The brief and unpredictable nature of the vase life of cut roses is mainly attributable to the early failure of tissue water relations, which is related to a decrease in water absorption due to vascular occlusion and the rapid loss of water from cut flowers under unfavorable postharvest conditions (van Doorn, 1989; Doi et al., 2000; Fanourakis et al., 2012). Although water relations are a major determinant of vase life in cut roses, it has been established that rose flowers are also susceptible to damage by ethylene, and produce substantial amounts of ethylene in response to various stress conditions, such as water deficit, vibration, darkness, high temperature, cold storage, or transport (Faragher et al., 1987; Mor et al., 1989; Muller et al., 2001a).

Ethylene regulates multiple aspects of plant growth and development, including flower opening, petal senescence and abscission, in a wide range of flower species (Abeles et al., 1992; Reid and Wu, 1992; van Doorn and Woltering, 2008). When ethylene is perceived by specific receptors, an ethylene signal is sent through a sequence of biochemical events that regulate the expression of ethylene-responsive genes, leading to ethylene synthesis and ultimately flower senescence (Woodson and Lawton, 1988; Borochov and Woodson, 1989; Verlinden et al., 2002). It has been established that there are two rate-limiting steps in the ethylene biosynthesis pathway: the conversion of *S*-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS), and the subsequent conversion of ACC to ethylene by ACC oxidase (ACO) (Yang and Hoffman, 1984). ACO1 has been shown to play a predominant role in *de novo* ethylene synthesis during senescence and its expression is rapidly induced in response to ethylene (Woodson et al., 1992; Muller et al., 2001b). ACS members

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http://dx.doi.org/10.1016/j.postharvbio.2017.05.003
Received 24 January 2017; Received in revised form 5 May 2017; Accepted 5 May 2017

appear to play different roles within specific tissues during the development and senescence phases of flowers (tenHave and Woltering, 1997; Jones and Woodson, 1999; Xue et al., 2008).

The sensitivity to ethylene is most probably mediated by changes in the ability to perceive ethylene during flower development (Bleecker et al., 2004). Although roses are classified as ethylene-sensitive (Woltering and van Doorn, 1988), sensitivity to the hormone varies considerably depending on cultivar (Reid et al., 1989a; Muller et al., 1998; Ichimura et al., 2002; Macnish et al., 2010). Thus, it is important to identify the degree of sensitivity and response to ethylene among rose cultivars and the senescence characteristics of cut flowers in response to ethylene.

In this study, we examined the ethylene sensitivity and senescence characteristics associated with the ethylene response of 33 rose cultivars. To gain a better understanding of flower senescence, we characterized the relationship between ethylene sensitivity and biosynthesis by determining the flower response to ethylene and the expression patterns of ethylene biosynthesis genes. The factors affecting the longevity of ethylene-sensitive and −insensitive cultivar groups were also analyzed to identify the contributions of ethylene damage and water stress to reduction in the potential vase life of cut roses.

2. Materials and methods

2.1. Plant material

Cut roses (*Rosa hybrida* L.) of 33 cultivars were obtained from two commercial growers in Gwangju (20 cultivars) and Goyang (6 cultivars) and a greenhouse (7 cultivars) of the Gyeonggido Agricultural Research & Extension Services in Korea. In all greenhouses, rose plants were grown using the "Arching" technique on rockwool slabs in natural light under drip irrigation with nutrient solutions. Cut flowers at the commercial maturity stage (onset of outer petal reflex) were harvested within a month in summer (July 7 and 21 and August 8 in 2016) to minimize the impact of variation in growth environments among the greenhouses. After harvest, cut flowers were immediately placed in a bucket containing tap water and transported within 2–4 h to the laboratory. The rose stems were trimmed to 40 cm with three upper leaves. The spray rose stems contained five florets with three upper leaves on the main stems. In all experiments, flowers were placed in a glass jar containing 500 mL distilled water and 3 replicates consisting of 3 flowers per vase (9 flowers) were used for each treatment.

2.2. Ethylene treatment

For ethylene treatments, cut flowers were enclosed in treatment chambers (462 L) at 22 ± 3 °C under dark conditions. Air circulation in the chambers was generated by a small fan. Ethylene was injected into the chambers to provide a final concentration of 10 μL L^{-1} and cut flowers were incubated under an ethylene atmosphere for 20 h. A beaker containing 100 mL of 1 M NaOH was placed in the chambers to prevent accumulation of CO_2 released by respiration during treatment. Untreated flowers were incubated in similar chambers with normal air. Ethylene treatment was terminated by transferring the flowers from the treatment chambers to air. For vase life evaluation, six flowers among the nine used for each treatment were placed in an environmental controlled room at 25 °C, 50% relative humidity, and 30 μmol m^{-2} s^{-1} of fluorescent lighting for 12 h. The remaining three flowers in each treatment were used for RNA isolation on first day after ethylene treatment.

2.3. cDNA synthesis

Outermost petals were detached from rose flowers on first day after ethylene treatment and on last day of vase life. These petals were immediately frozen in liquid nitrogen and then stored at −80 °C until RNA isolation. Three petals (~100 mg) were ground with liquid nitrogen to a fine powder using a pre-chilled mortar and pestle and total RNA was isolated from petal tissues using a Ribospin™ Plant kit (Gene All, Gene All Biotechnology Co., LTD, Korea), according to the manufacturer's procedure with slight modifications. Total RNA was quantified at 260 nm/280 nm using a NanoDrop spectrophotometer (NanoDrop Lite, Thermo Scientific, USA). Purified total RNA was treated with RNase-free water and used for cDNA synthesis using a Power cDNA Synthesis Kit (INTRON Biotechnology, Inc., Korea). First-strand cDNA was synthesized from 0.1 μg of total RNA using 1 μg of oligo$(dT)_{15}$ primer, 5 × RT buffer, dNTPs, DTT, RNase inhibitor, and AMV RT enzyme in a final volume of 20 μL, according to the manufacturer's instructions. Reverse transcription was performed in a SimpliAmp Thermal Cycler (AB Applied Biosystems, Singapore) for 5 min at 75 °C followed by 60 min at 42 °C.

2.4. Quantitative real-time PCR (qRT-PCR)

Gene-specific primers were designed for ethylene biosynthesis genes (*RhACS1*, *RhACS2*, *RhACS3*, *RhACS4*, and *RhACO1*) and synthesized by Cosmogenetech (Seoul, Korea). *Rosa hybrida* actin (*RhACT1*) was used as an internal control. The primer pairs used for qRT-PCR analysis are listed in Table 1. qRT-PCR was performed using the StepOnePlus™ real-time PCR system (Applied Biosystems, USA). Reaction mixtures contained 1 μL of cDNA as a template, 2 μL of 0.5 μM forward and reverse primers, and 10 μL of 2 × Maxima SYBR Green/ROX qPCR Master Mix (Applied Biosystems, USA) in a final volume of 20 μL dispensed in an optical 96-well plate. The qRT-PCR reactions were conducted using the following fast thermal cycle: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The threshold cycle (Ct) value was automatically detected for each reaction by the qRT-PCR system with default parameters. The final Ct value was the mean of three independent biological replicates and the coefficient of variance (CV) for each gene was also calculated. The relative level of gene expression was calculated as the absolute integrated absorbency normalized to that of actin.

2.5. Evaluation of vase life and senescence symptoms

Treatment effects among the 33 rose cultivars were determined by measuring changes in water uptake, relative fresh weight, and flower diameter daily at 09:00. Flower diameter was determined by measuring the largest diameter of the flower and the diameter perpendicular to it. Water balance was calculated by deducting daily transpiration from

Table 1
Gene-specific primers used for amplification of cDNA fragments by qRT-PCR.

Gene	Accession number	Forward primer	Reverse primer	Size
RhACO1	AF441282.1	5′-CGTTCTACAACCCAGGCAAT-3′	5′-TTGAGGCCTGCATAGAGCTT-3′	130
RhACS1	AY378152.1	5′-CAGTGAGAAAGGGGAGCTTG-3′	5′-TGTATTGAACCGGGATGGTT-3′	102
RhACS2	AY803737.1	5′-GCGAACAGGGGTACAACTTC-3′	5′-GGGTTTGAGGGGTTGGTAAT-3′	147
RhACS3	AY803738.1	5′-CAGTGAGAAAGGGGAGCTTG-3′	5′-AACCATCCCGGTTCAATACA-3′	142
RhACS4	AY525068.1	5′-GCTTCCAACTTGGGATCAAA-3′	5′-GCTCCATGAAACTTGCCATT-3′	100
RhACT1	KC514918.1	5′-GTTCCCAGGAATCGCTGATA-3′	5′-ATCCTCCGATCCAAACACTG-3′	116