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Potential vase life of cut roses: Seasonal variation and relationships with growth conditions, phenotypes, and gene expressions

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ABSTRACT

In this work, we identified the interrelationships between gene expression levels, environmental factors, phenotypic characteristics, and vase life of cut rose 'Lovely Lydia' between seasons and across years. We also determined the contribution of each factor to potential vase life (PVL) of cut roses. The vase life of the cut flowers was longest in spring (12.2 d), followed by summer (11.3 d), autumn (10.0 d), and winter (9.2 d). The environmental conditions in winter were characterized by high relative humidity (RH) and low vapor pressure deficit, and cut flowers grown under these conditions had less-functional stomata and consequently excessive water loss after harvest, resulting in shortened vase life. Leaf brix was not significantly correlated with PVL, but it was strongly correlated with flower diameter, indicating that congenital sucrose may be more important for providing the substances required for flower opening than for determining the longevity of cut roses. Correlation analysis revealed that initial transcript levels of the ethylene receptor *RhETR4* and the signaling component *RhEIN3-2* are very important factors in determining PVL of cut roses, as are preharvest RH conditions, stomatal function, and transpiration. The transcript levels of these genes were significantly modified by growth environment, including high RH and low temperature. Importantly, we show that RH conditions during cultivation not only determine stomatal characteristics but also modify the initial transcript levels of the ethylene responsive genes, thereby modifying the PVL. Understanding the interrelationships of genetic variation with other factors in the modulation of PVL will greatly help to improve growth environments and postharvest treatments across seasons as well as to develop techniques for guaranteeing the longevity of cut flowers.

1. Introduction

In the cut flower industry, quality assessment has generally been based on the appearance of the cut flowers, including flower size, color, and shape, as well as stem length and form (Reid, 2004; JETRO, 2011). Although the internal quality (i.e., longevity) is not currently incorporated into existing quality standards, the shorter longevity expected in cut flowers that are shipped overseas is often reflected in their prices in the international flower markets. Longevity is one of the most important criteria in consumer choice of cut flowers, and its short, unpredictable nature has resulted in low customer satisfaction (Reid, 2009; Rihn et al., 2014; MAFF, 2015). Customer satisfaction is extremely important for competing in the major flower markets such as the EU, the US, and Japan. Consequently, cut flower retailers worldwide are increasingly demanding guarantees of the potential vase life (PVL) on behalf of their customers (van Kooten and Kuiper, 2009; MPS-Japan, 2014). To guarantee the PVL for consumers, factors that influence flower longevity must first be quantified and then effective techniques to assess cut flowers should be developed.

Cut roses often end their vase life at an early stage of maturity due to petal withering, bending of peduncles (bent neck), and petal abscission, rather than undergoing the full natural senescence process like intact flowers do (Burdett, 1970; De Stigter, 1980; Zieslin, 1989; van Doorn, 2002). This short vase life of cut flowers is primarily attributable to the 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 leaves under unfavorable postharvest conditions (van Doorn, 1989; van Meeteren, 1992; Doi et al., 2000). The water relations of cut roses is closely related to their morphological and physiological characteristics such as leaf surface area, stomatal density, and stomatal function, and these are determined by the interactions of genotype with growth environmental conditions such as relative humidity (RH), vapor pressure deficit (VPD), and supplementary lighting in the greenhouse (Mortensen and Gislerod, 2000; Torre et al., 2003; Fanourakis et al., 2013). Previous studies have shown a strong relationship between the preharvest environment and variations in the vase life of cut roses even

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when the flowers are held under identical postharvest conditions (Mortensen and Gislerod, 2000; Marissen and Benninga, 2001; Slootweg et al., 2001; Pompodakis et al., 2005). Rose plants grown under high RH and low VPD conditions fail to regulate transpiration by attenuated stomatal function and exhibit considerable water loss after harvest, leading to shortening of the vase life (Torre et al., 2003; In et al., 2007; Fanourakis et al., 2012). Previously, we identified the quantitative impacts of preharvest environmental and phenotypic factors on the PVL of cut roses using multivariate analysis (In et al., 2007). The relationship between genotype and postharvest water relations and longevity have also been identified somewhat in cut roses (Mortensen and Gislerød, 1999; Ichimura et al., 2002; Fanourakis et al., 2012). However, variation in genetic characteristics and its relationship with the PVL of cut roses has not yet been fully addressed.

Despite the strong correlation of vase life with water relations, rose flowers are also susceptible to the plant hormone ethylene. They synthesize substantial amounts of ethylene in response to postharvest 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., 2000a; Macnish et al., 2010). Ethylene sensitivity is probably associated with changes in the ability to perceive ethylene (Bleecker and Schaller, 1996; Bleecker, 1999) and varies considerably depending on the variety and maturity of the flowers (Reid et al., 1989; Muller et al., 1998; Ichimura et al., 2002; Macnish et al., 2010; Borda et al., 2011). The ethylene effects on vase life and flower development of cut roses are mediated by expression of ethylene receptor and signaling genes such as *RhETR1*, *RhETR3*, and *RhCTRs* (Muller et al., 2000b, 2002; Ma et al., 2006; Xue et al., 2008). Moreover, expression of ethylene biosynthetic genes is positively correlated with ethylene synthesis in tissues. Therefore, it is important to identify the variation in expression patterns of ethylene-related genes and its relationship to PVL of cut flowers.

In this work, we determined gene expression levels, environmental factors, phenotypic characteristics, and the PVL of cut roses grown year-round in the greenhouse. The interrelationships between the factors obtained and the vase life was characterized by correlation analysis to identify the contribution of each factor to the longevity of cut roses. As part of this work, the transcript levels of ethylene biosynthesis and signaling genes were monitored to address ethylene impacts on the PVL of cut roses and to understand how the gene expression is changed by preharvest environments. Based on the results of the correlation analysis, the influence of primary factors on the PVL of cut roses was quantified by multiple regression analysis.

2. Materials and methods

2.1. Plant material and growth condition

The spray rose ‘Lovely Lydia’ (*Rosa hybrida* L.) flowers were obtained from commercial rose growers in Jangsu, Korea. The rose plants were grown in greenhouses on rockwool slabs using the “arching” method and were drip-irrigated with a nutrient solution containing 0.78 g L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 0.5 g L^{-1} NH_4NO_3, 0.17 g L^{-1} KH_2PO_4, 0.34 g L^{-1} KNO_3, 0.28 g L^{-1} $MgSO_4{\cdot}7H_2O$, and small amounts of other compounds. The temperature and RH were recorded at 30-min intervals with a data logger (WatchDog 1450, Spectrum Technologies, Aurora, IL, USA). The VPD was calculated from the temperature and RH data. Solar irradiation was measured by a radiation sensor (SQ-100; Apogee Instruments, Inc., Logan, UT, USA) connected to the data logger. The pH and the electrical conductivity (EC) of the nutrient solutions were measured with a pH meter (PH37-SS, Hach Company, Loveland, CO, USA) and an electrical conductivity meter (2265FS/2265FSTP, Spectrum Technologies), respectively. Values used for analysis were averages of the daily values for the 15 days before each harvest.

Thirty rose stems at an identical stage of maturity (onset of outer petal reflex) were randomly harvested from locations near the data loggers on January 29, March 26, April 20, May 10, June 23, July 13, August 17, October 30, and December 8 in 2015, and on February 16, March 17, May 10, July 25, October 5, and December 10 in 2016.

2.2. Morphological and physiological characteristics

After harvest, cut flowers were immediately placed in a bucket containing tap water and transported to the laboratory within 2 h. At the laboratory, the initial fresh weight, stem length, and stem diameter of the individual flowers were measured as the external quality (appearance) factors. The stem hardness, defined as the strength of the stem neck and the cut end, was measured with a digital hardness tester (TH200, Time Group, Beijing, China). Tissue samples (0.1 g) of the uppermost leaf were ground using a TissueLyser (TissueLyser II; Qiagen, Hilden, Germany) with 0.5 mL distilled water, and the soluble solids (brix) content was measured with a portable refractometer (PR-104, Atago, Tokyo, Japan).

The day after harvest, leaf stomatal sizes and densities were measured in the dark (after 12 h in dark conditions) and in the light (after 1 h at 30 μmol m^{-2} s^{-1}). The stomatal size and number on the abaxial surface of the uppermost leaf were measured with Suzuki’s Universal Micro-Printing (SUMP) method. Impressions of the leaf surfaces were photographed with a digital camera (PL-A662, PixeLink, Ontario, Canada) that was connected to an optical microscope (BX51; Olympus, Tokyo, Japan). The length, width (except the guard cells), and number of stomata were analyzed from the images using Image J software (Version 1.49p, NIH, Bethesda, MD, USA). The stomatal size was calculated using the following equation:

$$\text{Stomatal size} = \pi \times r_1 \times r_2$$

where r_1 is half of the length and r_2 is half of the width.

2.3. Vase life evaluation

Among the thirty cut spray roses, twelve were selected for evaluation of vase life. The stems were re-cut to a length of 50 cm; each stem contained five florets with three upper leaves on the main stems. Each cut stem was placed through a hole (1 cm diameter) in the center of the cap on a glass jar containing 500 mL distilled water, which was subsequently maintained in a controlled-environment room at 25 °C, 50% RH, and a photoperiod of 12 h with light supplied by fluorescent tubes at 20 μmol m^{-2} s^{-1} light intensity.

Variation in postharvest quality of cut roses between seasons was 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 daily water uptake.

The vase life was determined as the time from the placement of the cut flower vases in the environmentally controlled room to the end of vase life. Assessments of vase life were performed daily in accordance with the evaluation card for Rosa (VBN, 2014) with modifications. Briefly, roses were considered to have reached the end of their vase life when one or more of the following senescence symptoms was detected in at least three of the five florets: bending of the pedicel (bent-neck; neck angle greater than 45°), wilting (≥ 50% petal turgor loss), bluing (≥ 50% blue petals), petal abscission (drop of three or more petals), and leaf abscission and yellowing (≥ 50% leaf drop and yellowing).

2.4. Petal color, leaf chlorophyll, and bacterial contamination

The change in petal color during the vase life of the cut roses was determined daily using randomly selected outermost petals. The petal color was measured with a chromameter (CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) using CIE-L*a*b* coordinates, hue (h* ab),