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Nano-silver pulse treatments improve water relations of cut rose cv. Movie Star flowers

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ABSTRACT

Effects of pulse treatments with nano-silver (NS) particle (2–5 nm diam) solutions on the vase life of cut rose cv. Movie Star flowers were investigated. Pulse treatments of NS at 50, 100 and 250 mg L^{-1} were for 1 h. Stems were then transferred to deionized water (DI) and evaluated daily for vase life and quality. The 250 mg L^{-1} NS pulse treatment was phytotoxic. However, pulse treatments for 1 h with 50 and 100 mg L^{-1} NS solutions extended vase life and suppressed reduction in fresh weight during the vase period. The amounts of water uptake and water loss by the cut flowers decreased upon NS treatment. Stem hydraulic conductance decreased with time, but this decrease was suppressed by pulse treatments of 50 and 100 mg L^{-1} NS. ICP-AES analyses revealed that the Ag concentration in basal stem ends was generally higher than in upper stem ends, leaves and petals. NS pulse treatments reduced stomatal aperture and inhibited leaf transpiration. They also delayed expression of the aquaporin gene, *Rh-PIP2*. These evidently beneficial effects of NS pulse treatments are discussed.

1. Introduction

Water balance is a major factor determining quality and longevity of cut flowers. It is influenced by water uptake and transpiration, being the balance between these two processes (da Silva, 2003). When the amount of transpiration exceeds the volume of water uptake, water deficit and wilting develop (Halevy and Mayak, 1981). Low water uptake is often due to occlusions located mainly in the basal stem end (He et al., 2006), and microbes are a common cause of stem end blockage (van Doorn, 1997). Many agents have been used in cut flower vase solutions to extend vase life by improving water uptake. These include silver nitrate (Fujino et al., 1983), aluminum sulphate (Ichimura and Shimizu-Yumoto, 2007) and 8-hydroxyquinoline sulphate (Ichimura et al., 1999). Reducing transpiration can also improve the water balance of cut flowers and, thereby extend vase life. Ueyama and Ichimura (1998) reported that pulse treatments with 2-hydroxy-3-ionene chloride polymer (HICP) extended the vase life of cut roses by inhibiting transpiration from leaves and by its surfactant action. Tea Seed Saponins (TSS) as a surfactant also induced stomatal closure and extended vase life of cut rose flowers (Ichimura et al., 2005). Exogenous abscisic acid (ABA) is also effective in decreasing stomatal opening (van Doorn, 1997). However, while ABA treatment extended longevity of cut rose flowers held at relatively low relative humidity (RH) and due to stomatal closure, its supply accelerated senescence of roses held under relatively low evaporative demand (Halevy et al., 1974).

Compared with long distance water transport via the xylem system, short distance transport and transport in non-vascular tissues involves transport across membranes. Transmembrane water transport occurs by diffusion through the semi-permeable lipid bilayer and by transport through proteinaceous water channels, aquaporins (AQPs) (Johansson et al., 2000). AQPs are primary channels of water transport across biological membranes and are abundant in the tonoplast (vacuolar) membrane and the plasma membrane (Baiges et al., 2001). AQPs can increase the osmotic hydraulic conductivity of membranes by 10–20-fold (Preston et al., 1992). Plasma membrane Intrinsic Proteins (PIPs) are plasma membrane associated AQPs (Johanson et al., 2001). They are believed to play a role in many developmental processes in plants, including cell enlargement (Volkov et al., 2007), root hydraulic conductance (Gloser et al., 2007), organ movement and stomatal aperture change (Morillon and Chrispeels, 2001; Tyerman et al., 2002). Stomatal closure requires net efflux of K^+ at both the plasmalemma and the tonoplast of the guard cell. This results in loss of turgor, guard cell shrinkage and reduction in stomatal aperture (MacRobbie, 2006a,b). AQPs have a role in stomatal aperture changes (Sarda et

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al., 1997; Fraysse et al., 2005). In cut rose flowers, Ma et al. (2008) established involvement of a rose PIP gene, *Rh-PIP2*, in ethylene regulated petal expansion.

Nano-silver (NS) as a pulse and vase solution treatment for cut flowers is relatively new (Liu et al., 2009; Solgi et al., 2009) and has demonstrated importance as an antibactericidal agent (Alt et al., 2004; Morones et al., 2005). NS releases Ag^+ (Lok et al., 2007), which has been reported to interact with cytoplasmic components and nucleic acids, to inhibit respiratory chain enzymes and to interfere with membrane permeability (Russell and Hugo, 1994; Park et al., 2005). Use of NS is becoming increasingly widespread in medicine, fabrics, water purification and various other industrial and non-plant applications (Jain and Pradeep, 2005; Dubas et al., 2006; Chen and Schluesener, 2008). Liu et al. (2009) reported vase life extension for cut gerbera cv. Ruikou flowers following pulsing with 5 mg L^{-1} NS solution for 24 h. The positive effect of a NS pulse treatment was attributed to inhibition of bacterial growth in the vase solution and at the cut stem ends during the first 2 d of the postharvest period. However, physiological activity of Ag^+ from NS is also a possibility. Ag^+, generally applied as silver thiosulfate, effectively inhibits ethylene-mediated processes, such as flower senescence and abscission (Altman and Solomos, 1995; Ichimura et al., 2008). As with other cations (e.g. K^+, Ca^{2+}), positive effects on plant stem hydraulic conductivity of Ag^+ (van Ieperen, 2007) are possible. Also, Ag^+ is considered a general inhibitor of AQPs (Niemietz and Tyerman, 2002). Ohkawa et al. (1999) reported that silver-containing compounds extended the vase life of cut roses.

We investigated effects of NS on vase life, transpiration and stem hydraulic conductance of cut rose flowers. NS pulse treatments extended vase life, inhibited transpiration from leaves and suppressed the decrease in stem hydraulic conductance. NS treatments also reduced stomatal conductance and transpiration rate and increased water content in cut rose leaves. Expression of an aquaporin (AQP) gene, *Rh-PIP2*, in rose leaves was delayed by NS.

2. Materials and methods

2.1. Plant material

Cut rose (*Rosa hybrida* cv. Movie Star) flowers were purchased from a wholesale cut flower market in Guangzhou city, China. They were immediately stood upright in buckets partially filled with tap water and transported within 1 h to the postharvest laboratory at Zhongkai University of Agriculture and Engineering. Buckets containing the flower stems were covered with a plastic film shroud to minimize moisture loss during transportation. At the laboratory, stems were re-cut under deionized water (DI) to ~25 cm length. Recutting was to ensure no air blockage of the stem end. The flowers were selected for uniformity of size, colour and freedom from any defects. The upper two leaves were retained on each stem. They were pulse-treated for 1 h with NS (Shanghai Huzheng Nano Technology Co. Ltd., China) solutions at 50, 100 and 250 mg L^{-1} in a phytotron operating at 20 ± 2 °C, 60 ± 10% RH and 12 μmol m^{-2} s^{-1} light intensity (cool white florescent tubes) under a daily light period of 12 h.

2.2. Experimental design and treatments

All experiments were conducted in the phytotron as described above. In each experiment, cut rose stems were placed individually into 180 mL glass vases containing 150 mL of DI water as the vase solution. Mouths of the vases were covered with a sheet of low density polyethylene film to minimise evaporation and prevent contamination. Vases were arranged on benches in a randomized complete block design. All solutions were freshly prepared at the beginning of the experiments and were not renewed in the course of the experiments.

2.2.1. NS pulse treatments

NS pulse treatments were carried out using previously published methods (Liu et al., 2009), with slight modifications. Three concentrations (50, 100 and 250 mg L^{-1}) of NS for pulse treatments were used in experiments. Control stems were pulsed with DI water. All pulse treatments were for 1 h in the phytotron. Treated stems were immediately stood in vases containing DI water.

Vase life was the period from the time of harvest to the time when petals lost turgor, necks (peduncle) were bent or petals had abscised. Water uptake, water loss and fresh weight were recorded daily by measuring weights of vases without flowers and of flowers separately. Average daily water uptake was calculated as: water uptake (g $stem^{-1}$ d^{-1}) = $(S_{t-1} - S_t)$; where, S_t is weight of vase solution (g) at t = days 1, 2, 3, etc., and S_{t-1} is weight of vase solution (g) on the previous day. Average daily water loss was calculated as: water loss (g $stem^{-1}$ d^{-1}) = $(C_{t-1} - C_t)$; where, C_t is the combined weights of the cut stem and vase (g) at t = days 1, 2, 3, etc., and C_{t-1} is the combined weights of the stem and vase (g) on the previous day. Water balance was calculated as water uptake from the vase minus water loss from the stem. Relative fresh weight (RFW) of stems was calculated as: RFW (%) = $(W_t/W_{t-0}) \times 100$; where, W_t is weight of stem (g) at t = days 0, 1, 2, etc., and W_{t-0} is weight of the same stem (g) at t = day 0 (He et al., 2006).

2.2.2. Leaf water content

Leaf dry weights (DW) were recorded after drying to constant weight in an oven for at least 96 h at 62 °C (He et al., 2006). Water content was calculated as (FW − DW)/DW (Jones et al., 1993). Water content was determined on days 0, 1, 4, 7 and 10 for three replicate detached leaflets from different stems.

2.2.3. Stem hydraulic conductance

Hydraulic conductance of stem segments was measured as described by He et al. (2006). The basal 2 cm of the stem was inserted into a silicon tube (ID 4 mm) and a 100 cm head pressure of 10 mg L^{-1} sodium dichloroisocyanurate (DICA) solution (10 kPa) was applied. The DICA solution that passed through the segments was collected in vessels. The amount of the collected solution within 24 h was measured by electronic balance (A&D Company Limited, Japan). Each experiment was repeated three times.

2.2.4. Bacterial counts

For determination of numbers of bacteria in stems of the different treatments, 2 cm length (~0.5 g) segments were cut from the stem ends. These explants were washed three times with sterile DI to reduce the surface load of microbes. They were then ground and diluted with 0.9% sterile normal saline. Liquid extract (0.1 mL) was spread on nutrient agar plates and bacterial colonies were enumerated after incubation for 24 h at 37 °C. All bacteria counting was replicated three times (Balestra et al., 2005).

2.2.5. Stomatal conductance and transpiration rate

Stomatal conductance and transpiration of the uppermost leaves was measured with a portable photosynthesis system (LI-6400XT, LI-COR, USA). Determination of stomatal conductance and transpiration were according to the instrument's own formulas.

2.2.6. Scanning electron microscopy (SEM)

Rose leaf stomata were examined by SEM. Small area leaf samples (~2 mm × 5 mm) from the apex were taken from the cut roses at 3 pm on days 0, 3, 5 and 7, and immediately placed into a fixative mixture of 4% glutaraldehyde in phosphate buffer (pH 6.8)