WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
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Journal of Food, Agriculture & Environment Vol.8 (2): 1118-1122. 2010 *www.world-food.net*

Effects of nano-silver treatment on vase life of cut rose cv. Movie Star flowers

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Received 9 February 2010, accepted 5 April 2010.

Abstract

Vase life of cut roses cv. Movie Star placed in deionized water was only 9 to 10 days regardless of the maturity at harvest. In the present study, the causes of short vase life for cut roses and the effects of treatments prolonging vase life were investigated in relation to water stress and bacterial contaminations. Flowers of cut rose cv. Movie Star were pulsed in nano-silver (NS) base solution for 24 h and then kept in a low concentration NS solution. Effects on vase life and the flower quality were evaluated by analyzing vase life, water uptake, water loss, changes in stomatal conductance and scanning electron microscopic observation of stem. The pulse treatment in 10 mg L^{-1} NS plus 5% sucrose solution for 24 h followed by holding samples in 0.5 mg L^{-1} NS plus 2% sucrose solution not only alleviated and delayed vascular blockage caused by bacterial contaminations but also inhibited stomatal conductance so that the water balance in cut roses was significantly improved and the vase life of the cut rose flowers was prolonged by 11.8 days.

Key words: Cut rose, vase life, nano-silver, anti-microbial, sucrose, water relations.

Introduction

Roses are important cut flowers and many studies have therefore focused on their quality after harvest [1]. The vase life is often very short characterized by early wilting and bending of the pedicel. The development of such symptoms is considered to be caused by vascular occlusion [2], mainly located in the basal stem end [3, 4]. Microbial contaminations are thought to be the most common cause of stem blockage [5, 6]. Thus, many beneficial agents, such as silver nitrate [7], aluminium sulphate [8, 9] and 8-hydroxyquinoline sulphate [10], have been used in vase solution for cut flowers to extend vase life by improving their water uptake. Otherwise, reducing transpiration could also improve water balance of the cut flower and extend the vase life. A previous study [11] reported that pulse treatment with 2-hydroxy-3-ionene chloride polymer (HICP) extended the vase life of cut roses by inhibiting transpiration from leaves and the surfactant action. Tea-seed saponins (TSS) as surfactant also induced stomatal closure and extend vase life of cut rose flowers [12]. Cycloheximide extended vase life of cut *Iris* flowers by 4 days by inducing rapid stomatal closure and decreases in transpiration rate and water uptake [13].

Nano-silver (NS) as germicide is relatively new. Many studies have demonstrated the importance of NS (Ag^+) particles as an antibacterial agent [14-17]. The antibacterial activities of NS are related to their size, with higher surface to volume ratio and increased percentage of atoms at the grain boundaries [18], but little is known about their physiological effects on plants. Liu *et al.* [19] reported vase life extentions in cut gerbera cv. Ruikou flowers pulsed for 24 h in the 5 mg L^{-1} NS solution, by inhibiting bacteria growth at cut stem end. However, similar and modified studies are needed to understand the effects of NS pulse treatment on vase life of cut roses. Therefore, in the present research we conducted an experiments to elucidate the effects of NS on the vase life of cut rose flowers. Moreover, solutions of sugars such as sucrose or glucose are known to be important for flower vase life and it is a common practice to supply exogenous carbohydrates to cut flowers to increase their longevity [20]. There are also reports showing vase life extending effect of adding sugars to vase solution of cut flowers [10, 21, 22]. Thus, in the present study, effect of sugar addition on vase life of cut roses was also confirmed.

Materials and Methods

Cut rose cv. Movie Star flowers were purchased from a wholesale market in Guangzhou, China. Flowers were immediately placed upright in a bucket partially filled with tap water and transported within 1 h to the postharvest laboratory at Zhongkai University of Agriculture and Engineering. In the laboratory, the flower stem was re-cut under deionised water (DI), remaining approximately 25 cm long. This procedure aimed at eliminating air blockage in the stem. The flowers were selected for uniformity of size, color and free from any defects, each with uppermost 2 leaflets. All experiments were performed in a phytotron at temperature of 20 ± 2°C, relative humidity of 60 ± 10%, and light intensity of 12 µmol m^{-2} s^{-1} from cool white florescent tubes with a daily light period of 12 h. Solutions were freshly prepared at the beginning of experiments.

Experiment 1: Three treatments were designed in Experiment 1. Treatment 1 - NS-treated stems were kept for 24 h in 10 mg L^{-1} of NS (Shanghai Huzheng Nano Technology Co. Ltd., China) for pulsing, followed by holding the sample in 0.5 mg L^{-1} NS; Treatment 2 - Pulse treatments with 10 mg L^{-1} NS plus 5% sucrose solution

for 24 h followed by holding the samples in 0.5 mg L^{-1} NS plus 2% sucrose solution; and Control - Deionized water. For subsequent vase life assessment, rose stems were held individually in 200 ml glass vases each containing 150 ml of vase solution. The vase mouth was covered with a sheet of low density polyethylene film to minimize evaporation and prevent contamination. Vases were arranged on benches in a randomized complete block design. Vase life, water uptake and relative fresh weight of cut roses were assessed daily during the vase period. Eight flowers were used for each treatment. Vase life was the period from the time of harvest to the time when either the petals lost turgor, the necks were bent or the petals had abscised. The fresh weight of cut flowers and the amount of water uptake were measured daily. The weight of vases without and with the flower was separately recorded daily. Average daily water uptake was calculated as water uptake (g $stem^{-1}$ day^{-1}) = (S_{t-1}-S_t), where, S_t is weight of vase solution (g) at t = day 1, 2, 3, …, n, and S_{t-1} is weight of vase solution (g) on the previous day. Average daily water loss was calculated as water loss (g $stem^{-1}$ day^{-1}) = (C_{t-1}- C_t), where, C_t is the combined weights of the cut stem and vase (g) at t = day 1, 2, 3, …, n, and C_{t-1} is the combined weight of the stem and vase (g) on the previous day. The 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})×100, where, W_t is weight of stem (g) at t = day 0, 1, 2…, n, and W_{t-0} is weight of the same stem (g) at t = day 0 [4].

Experiment 2:

Determination of antibacterial zone: Plates of nutrient agar (Guangdong Huankai Microbial Sci. & Tech. Co. Ltd., China) each containing 0.1 ml vase solution of Control from the 10th day were prepared. Filter paper discs with 5 mm diameter pre-immersed in either 0.9% normal saline (as control) or 10 mg L^{-1} NS solution for 10 min were placed in the middle of the plates. The inoculated plates with discs were incubated at 37°C for 24 h and then the diameter of the clear, bacteria-free zone was recorded.

Microscopic observation: Stem cross-segments 3 mm long were sampled at the base of each stem for microscope observation immediately after cut and on the 5th day of the vase period. Each stem segment was immediately fixed in FAA, aspirated overnight and dehydrated. Following paraffin infiltration, serial sections of 10 µm were cut, mounted on glass slides, and finally coated in gold and examined at 10 kV using a JSM-6360LV SEM (JEOL, Japan) and photographed.

Determination of leaf stomatal conductance and transpiration rate: Stomatal conductance and transpiration of the uppermost leaves from the apex were measured by a portable photosynthesis system (LI-6400xt, Li-Cor, Inc., Lincoln, NE, USA) according to the instruction manual on day 0, 1, 3, 5, 7 and 12.

Statistical analysis: Experiments involved 3 to 8 replicates cut flowering stems for each treatment (see individual tables and figures). Data were subjected to analysis of variance (ANOVA) using the General Linear Model program of Minitab® Release 15. Means were compared by the least significant difference (LSD) test at the 0.05 probability level.

Results

Vase life: There were significant differences in vase life between NS-treated and control flowers. Treatment 1 extended the vase life of cut roses by 3.8 d. Treatment 2, with sucrose added, extended vase life to more than twice that of the control (Table 1). Moreover, NS treatments, especially Treatment 2, prevented petals and leaves from abscission and maintained their color in vase period.

Table 1. The effects of NS treatments on the vase life of cut rose cv. Movie Star (each value represents a mean ± SE of 8 replicates.

Treatments	Vase life (d)
Control	10.1 ± 0.94
Treatment 1	13.9 ± 2.31
Treatment 2	21.9 ± 0.93
LSD 0.05 (n=8)	2.26

Relative fresh weight: The changes in relative fresh weight (RFW) of cut roses in both Control and NS treatments showed similar trends, namely the RFW increased initially after harvest and decreased thereafter (Fig. 1). However, both NS treatments increased RFW at a rate larger than that in Control and began to decrease fresh weigh on day 5, 1 day delayed, compared with Control.

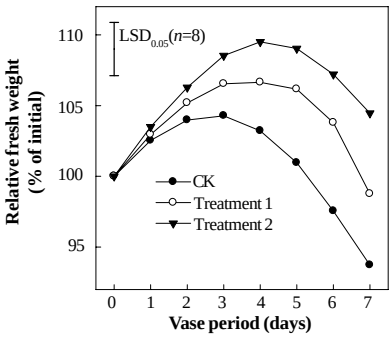

Figure 1. Effects of NS treatments on the relative fresh weight of cut rose (n = 8).

Water uptake, water loss and water balance: As shown in Fig. 2, water uptake or water loss in control flowers was greater than in flowers treated with NS. Water uptake in control flowers tended to increase on the first day, decreased on the 6th day and remained almost constant thereafter (Fig. 2A). Water loss increased with time in all flowers examined. Water loss in flowers treated with NS tended to increase steadily until day 7, still significantly lower than in the control flowers (Fig. 2B). Water balance is influenced by water uptake and water loss. In this study, the water balance declined almost linearly with vase time. Treatment 2 was the most effective in water balance regulation. Treatment 1 was also effective. In contrast, the water balance in control flowers declined faster than in NS treatments throughout the vase life period (Fig. 2C).

Antibacterial zone: Mean diameter of antibacterial zones around the 10 mg L^{-1} NS-saturated paper discs on agar was 11.39 mm. There was no antibacterial zone in the control (Fig. 3).

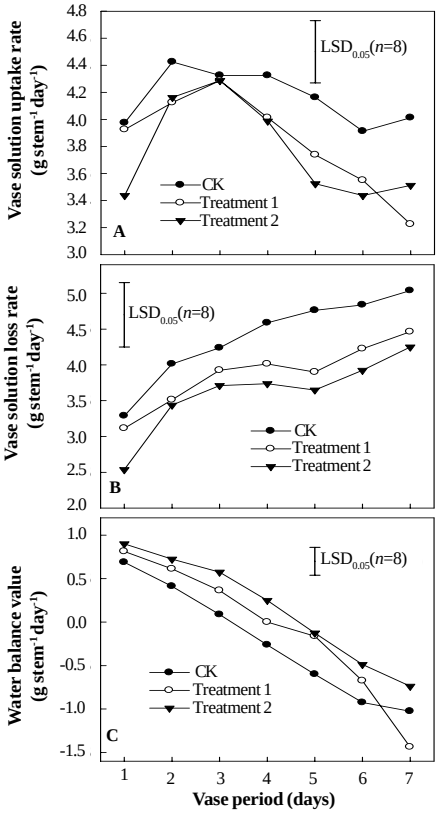

Figure 2. Effects of NS treatments on the vase water uptake (A), vase water loss (B) and vase water balance (C) of the cut rose.

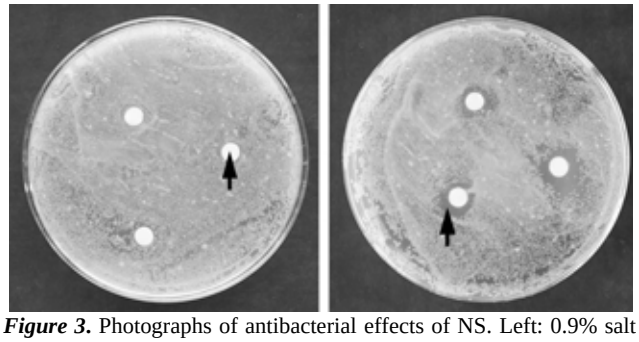

Figure 3. Photographs of antibacterial effects of NS. Left: 0.9% salt water (arrow showing the paper disc); Right: 10 mg L^{-1} NS (arrow showing the antibacterial zone).

Microscopic observation: High density visible microorganisms were observed 5 d after pulse treatment in the vessels of control roses (Fig. 4A). In contrast, only very few microorganisms were evident on day 5 in the vessels of cut roses treated with 10 mg L^{-1} NS (Treatment 2, Fig. 4B).

Leaf stomatal conductance and transpiration rate: After cut, water loss in rose leaves decreased sharply due to stomatal closure. Leaf stomatal conductance and the transpiration rate in NS treatments declined faster than in control (Fig. 5). Stomatal conductance in all leaves declined on the first day while showed an increase on day 3 and then declined thereafter. Treatment with NS decreased leaf stomatal conductance (Fig. 5A). Leaf

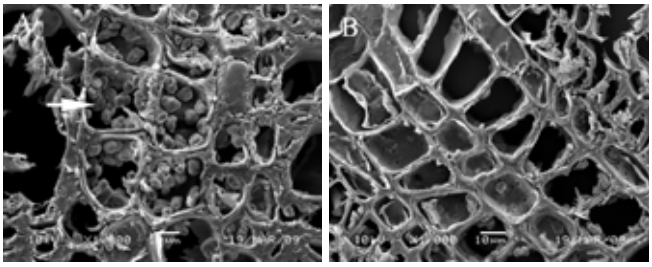

Figure 4. The cross section of the vessel blockage in the stem end of the cut rose flowers under SEM. The blockage in vessels of Control on day 5 (A); the blockage in vessels of Treatment 2 on day 5 (B) (white arrows showing the blockage in vessels).

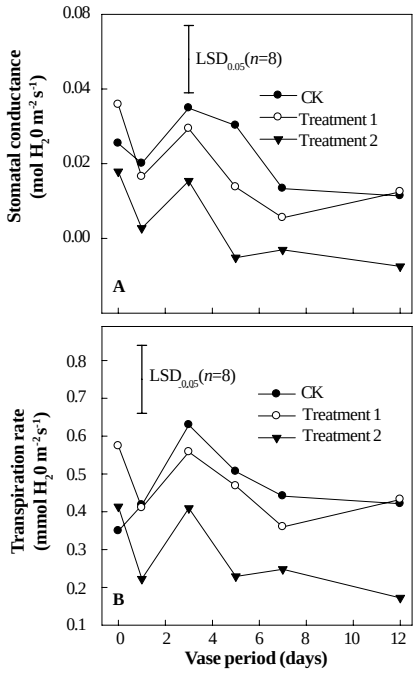

Figure 5. The effects of NS treatments on the stomatal conductance (A) and transpiration rate (B) of cut rose cv. Movie Star flowers ($n = 8$). Vertical bars indicate $LSD_{0.05}$ for the treatment by time interaction.

transpiration rate decreased on the first day and remained almost constant thereafter with the exception that it tended to decrease on the third day (Fig. 5B).

Discussion

Nanometer-sized silver (Ag^{+}) particles (NS) are recognized as having a stronger effect of inhibiting many bacterial strains and other microorganisms than silver in various oxidation states (Ag^{0}, Ag^{+}, Ag^{2+}, Ag^{3+}) because of their unique and high surface area-to-volume ratio [23, 24]. As a new antibacterial agent, NS is widely used in medicine, fabrics, water purification and related applications [25-27]. Liu *et al.* [19] reported that NS pulse treatment inhibited bacteria growth in the vase solution and at cut stem ends of cut gerberas. In this study, applications of NS to cut roses showed significant effect in extending vase life (Table 1), partly because of its actions of inhibiting bacteria growth in the vase solution (Fig. 3) and at cut stem ends (Fig. 4) during vase period. The present results with NS treatments were somewhat similar to

those with the cut gerberas, where NS inhibited bacterial growth and thereby extended vase life [19].

It is well known that exogenous supply of sugars, such as sucrose or glucose solutions, delays wilting in many flowers. The basis for this improvement is not fully understood but it is likely that sucrose supplementation is effective at a number of levels. Sucrose can act as an energy source [28] and osmotic regulator [29], thereby playing a role in flower opening and subsequent water balance regulation [30]. However, sugars in the vase solution, if not accompanied by an adequate antibacterial agent, may promote bacterial growth, and therefore inhibit the uptake of both water and dissolved sugars by impeding xylem vessels [31, 32]. Therefore, antibacterial agent plus sucrose is included in most cut flowers preservative formulations [33, 34]. The optimal concentration of sucrose varies with the treatment and the flowers. In general, high concentrations are used for pulsing and low for holding solutions [35]. Liu *et al.* [36] reported that NS treatments in combination with other solution components, such as sugar and quaternary ammonium compound, could further prolong the vase life of cut roses. In the present study, Treatment 1 without sucrose also supported a much longer vase life than control, but the vase life was not as long as that in treatment with NS plus sucrose (Table 1). NS played the role as an antibacterial agent (Figs 3 and 4) and therefore made full advantages of sucrose in extending vase life of cut roses [36].

A water deficit may develop only when the rate of water uptake is lower than the rate of transpiration, and a high rate of transpiration disrupts the water balance, which may then shorten the vase life of cut roses. Hence, the onset of water stress can be delayed by reducing water loss [31]. In the present experiment, treatments with NS decreased water loss and maintained optimal water balance (Fig. 2C) and therefore extended the vase life of cut rose flowers (Table 1). Further examinations showed that stomatal conductance and transpiration rate of the cut roses were found to be decreased by NS treatments (Fig. 5), probably due to stomatal closure induced by NS.

Many chemicals, such as abscisic acid [37], cycloheximide [13], 2-hydroxy-3-ionene chloride polymer (HICP) [11] and tea-seed saponins (TSS) [12], are used in vase solutions of cut flowers to improve the quality and prolong vase life by inhibiting transpiration, but most of these chemicals do not provide bactericidal activity. Our results showed that NS inhibited microbial growth and consequently decreased transpiration rate. In addition, combined application of NS with sucrose improved vase life, which was longer than control or NS alone in rose flowers (Table 1). This effect is probably attributed to supply of energy source by sucrose and regulation of water relations by NS. This suggests that treatment with NS plus sucrose can be practically useful in improving vase life of cut rose flowers, as a potential postharvest technology for many cut flowers.

Acknowledgements

This research was supported by grants from the Natural Science Foundation of China (No. 30771519), the Natural Science Foundation of Guangdong Province (No. 8251022501000002), and the Science and Technology Foundations of Guangzhou, China (No. 2007Z2-E0071 and No. 20082110021).

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