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NANO-SILVER PULSING AND CALCIUM SULFATE IMPROVE WATER RELATIONS ON CUT GERBERA FLOWERS

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ABSTRACT. *The studies showed nano-silver (NS) as antimicrobial agent can produce the valuable results to extend vase life of gerbera cut flowers. The aim of this study was to use NS with calcium sulfate (CS) and gibberellin$_{4+7}$ (GA) to evaluate their interactions. Pulse treatment of flowers were performed in NS (3 and 9 mg L^{-1}) solution and deionized water (DI), for 24 h. Then, flowers were treated with preservative solutions containing calcium sulfate (0, 10 and 20 mM) and GA_{4+7} (0 and 20 mg L^{-1}), and 1.5% sucrose were added to preservative solutions. Pulse treatments with 3 or 9 mg NS L^{-1} by holding in solution containing 20 mM CS compared to control treatment (pulsed in DI and held in solution of sucrose) increased total water uptake and water loss and extended vase life. Regression analysis showed that total water loss (independent variable) affect on vase life (dependent variable). Our results suggest application of CS (20 Mm) in vase solution after pulse treatment with NS.*

KEYWORDS**:** *calcium ions, cut flowers, silver nano-particles, transpiration, water uptake*.

INTRODUCTION

The world flower trade knows gerberas (*Gerbera jamesonii*) well for the variety of their colors, and popular (Liu et al. 2009a, Solgi et al. 2009).

However, often the growers and florists sustain a loss from short vase life of gerberas. The criteria used to determine end of gerbera vase life are the drooping of flower (stem bending; ≥ 90°) and/or moderate wilting of petals and/ or breaking (Geraspolus & Chebli 1999, Macnish et al. 2008, Solgi et al. 2009).

Whereas, gerberas are ethylene insensitive, therefore, bacterial plugging of the xylem is a main cause of early and rapid cut flower senescence (Liu et al. 2009a). The decrease of water uptake and the increase of transpiration (i.e. high value of water balance) will cause the xylem obstruction, and will end cut flower vase life. NS pulse and continuous treatments for cut flowers are newly using. It can use NS as the novel agent of anti-microbial (Liu et al. 2009a, Solgi et al. 2009, Lü et al. 2010). NS release Ag^{+} (Lü et al. 2010) that is destructive for the fungal and bacterial agents (Liu et al. 2009a, Solgi et al. 2009, Lü et al. 2010, Nair et al. 2010, Sharon et al. 2010). Naghsh (2010) explained the inhibition of NS on meiosis in *Aspergilus niger*. Alavi and Dehpour (2010) reported that nanosilver solution is effective on greenhouse cucumber downy mildew disease. Liu et al. (2009a) and Lü et al. (2010) reported NS pulse treatments extended vase life of cut gerbera and *Rose* flowers, respectively. In addition, Solgi et al. (2009) suggested, the sucrose should be used with antimicrobial agents e.g. NS for inhibition of the growth of bacteria in the vase solution and xylem vessels of stem, however this proposed mechanism of action (antimicrobial action) needs further experiments.

Calcium increases postharvest longevity of fresh cut flowers (Geraspolus & Chebli 1999, De Capdeville et al. 2005, Sosa Nan 2007). This increased postharvest longevity may be due to a delay of physiological events related to senescence, such as a decrease in water uptake, increased water transpiration loss, decreased fresh weight, and stem bending (Sosa Nan 2007). It seems that calcium ions affect ethylene action on cell membranes by inhibiting ion leakage, and reducing the effect of ethylene on senescence (De Capdeville et al. 2005). As mentioned before, gerbera are insensitive to ethylene, so calcium accumulation facilitates cross-linking of pectic polymers, particularly of the middle lamella, to form a cell wall network that increases mechanical strength (Geraspolus & Chebli 1999).

In addition to sucrose act as a food source in the preservative solutions, also it maintains water balance (Solgi et al. 2009). In addition, since the level of soluble carbohydrates will maintain by the treatment of gibberellin (GA) (Ranwala & Miller 2000, Whitman et al. 2001, Hatamzadeh et al. 2010), therefore, GA can affect on the water balance.

In the previous researches by others, the effects of NS pulse treatments were investigated on the vase life of cut gerbera, but interaction calcium sulfate and gibberellin continuous treatments with NS pulse treatments are not studied on the water relations and vase life of cut gerberas.

MATERIALS AND METHODS

Plant material

In this experiment, we used cut gerbera (*Gerbera jamesonii* cv. Pink Elegance) flowers grown in standard hydroponic greenhouse conditions and harvested the flowers by pulling out the stems from the plants when 2–3 rows of stamens of the bisexual disc florets were mature (Geraspolus & Chebli 1999, Solgi et al. 2009) at morning (October, in 2011). We put the end of harvested flowers stem on flower capsule filled with deionized water (DI). Flowers were packed and transported within 8 h to Faculty of Agriculture, University of Guilan, Iran. In the laboratory, stems were cut to a length of 45 cm under deionized water for removing air emboli (Liu et al. 2009a, Solgi et al. 2009). The stems end were re-cut twice or thrice at vase period.

The experiment was performed in a controlled room at 20 ± 2 °C, 60 ± 10% R.H., and 12 μmol m^{-2} s^{-1} light intensity (cool white fluorescent lamps) under a daily light period of 12 h.

Experimental design and treatments

Solutions of pulse treatments were prepared at the previous day of experiment in two concentrations of NS (3 and 9 mg L^{-1}; Nanonasb-Pars Company, Iran) and DI for 24 h. (). Each pulse treatment was comprised 54 flowers. After pulse treatment, flowers were kept individually into 1000 ml glass bottles containing 500 ml of fresh solutions that were prepared at the beginning day 2nd of experiment and were not renewed again. In continuous treatment, three concentrations (0, 10 and 20 mM) of calcium sulfate, CS, ($CaSO_4.2H_2O$, Merck Company; soluble in 1.9 M HCL, with a little heating), and two concentrations (0 and 20 mg L^{-1}) of GA_{4+7} (Serva Company, USA) were used. In the continuous treatments, sucrose 1.5% was applied. The control treatment was the pulsing in DI and the holding in sucrose solution.

Vase life was the period from the time of harvest to the time when flowers were showing symptoms of petal wilting or curling, stem bending (≥ 90°C) or breaking. Water uptake, water loss and fresh weight were recorded every other day by measuring weights of vases without flowers and of flowers separately. Average alternate day water uptake was calculated as: water uptake (g $stem^{-1}$ 48 h^{-1}) = ($S_{t-1} - S_t$); where, S_t is weight of vase solution (g) at t = days 0, 2, 4, etc., and S_{t-1} is weight of vase solution (g) on the previous day (He et al. 2006, Lü et al. 2010). Average alternate day water loss was calculated as: water loss (g $stem^{-1}$ 48 h^{-1}) = ($C_{t-1} - C_t$); where, C_t is the combined weights of the cut stem and vase (g) at t = days 0, 2, 4, etc., and C_{t-1} is the combined weights of the stem and vase (g) on the previous day (Lü et al. 2010). Relative fresh weight (RFW) of stems was calculated as: RFW (%) = (W_t / W_{t0}) ×100; where, W_t is weight of stem (g) at t = days 0, 2, 4, etc., and W_{t0} is weight of the same stem (g) at t = day 0 (He et al. 2006, Lü et al. 2010).

There were three replications and three samples per treatment in a completely randomized design as factorial experiment. Each sample was one flower per bottle. Data were analyzed using analysis of variance (PROC GLM), and the means were compared by Tukey's Test (HSD) at $P \leq 0.05$ using SAS (9.1) statistical software. Regression analysis (path analysis) was taken to determine the major factors that affect vase life (dependent variable). The independent explanatory variables were total water uptake, total water loss and total water balance. The software used for path analysis was SPSS/PC^+ "Stepwise" (version 11.5).

RESULTS

Interactions NS and CS extended the vase life (Figure 1). NS pulse treatments followed by holding in preservative solutions containing concentrations 10 or 20 mM CS extended the vase life compared to DI pulsing and preservative solution containing sucrose (N0C0, control treatment) (Figure 1). However, no significant ($P \leq 0.05$) differences were found among various concentrations of NS and CS in extending vase life.

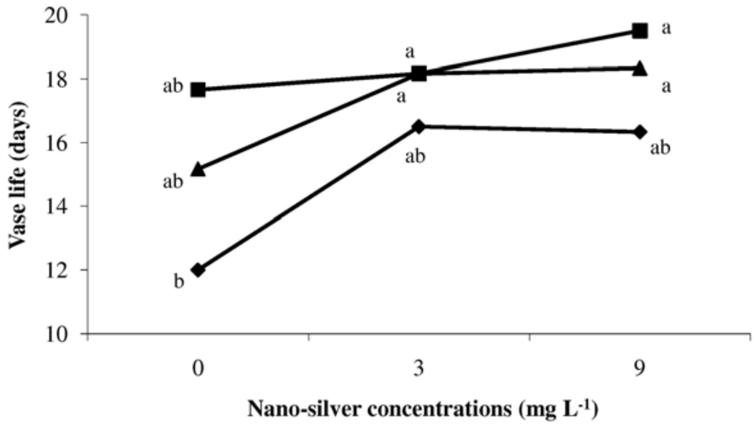

Figure 1. Interaction of nano-silver (0, 3 or 9 mg L^{-1}) and calcium sulfate concentrations (◆: 0 mM; ■: 10 mM; ▲: 20 mM) on the vase life (days) in cut gerbera flowers. The means with similar letters have no significant differences (P< 0.05).

Furthermore, interactions CS and GA_{4+7} were significant to extend the vase life (Figure 2). According to Figure 3, interactions NS and CS concentrations on the total water uptake with flower stems were significantly ($P \leq 0.05$) differed.

Total water loss was significantly increased when were applied either concentrations of NS (9 or 3 mg L^{-1}, respectively) followed by continuous treatment with CS 20 mM compared to DI pulsing followed with CS 20 mM (Figure 4). In addition, we observed this significant difference in the DI pulsing followed with CS 10 Mm. Percentage of increasing was 72.92, 66.63 and 63.21%, respectively. The coefficient of multiple determinations (R^2) was 0.236 in linear model for the vase life (Table 1). This coefficient gives the proportion of the total variation in the dependent variable (vase life) explained by the predictors included in the model. Thus, from among two independent variables, total water loss explained 23.6% of the observed total variation in the vase life, and other independent variable (total water uptake) had a lesser role in the vase life. Furthermore, the test statistic in linear model showed that coefficient of total water loss positively and significantly influenced vase life ($P \leq 0.05$). This effect needs further investigations.

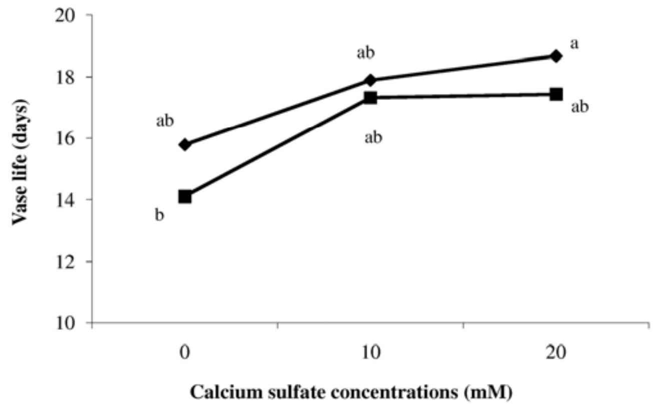

Figure 2. Interaction of calcium sulfate (0, 10 or 20 mM) and GA_{4+7} concentrations (◆: 0 mg GA_{4+7} L^{-1}; ■: 20 mg GA_{4+7} L^{-1}) on the vase life (days) in cut gerbera flowers. The means with similar letters have no significant differences ($P < 0.05$).

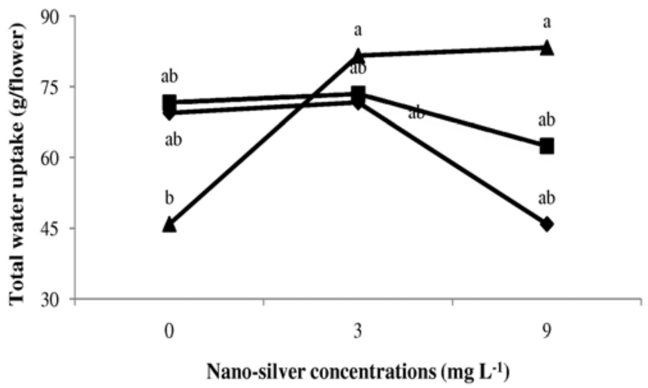

Figure 3. Interaction of nano-silver (0, 3 or 9 mg L^{-1}) and calcium sulfate concentrations (◆: 0 mM; ■: 10 mM; ▲: 20 mM) on the total water uptake (g/flower) in cut gerbera flowers. The means with similar letters have no significant differences ($P < 0.05$).

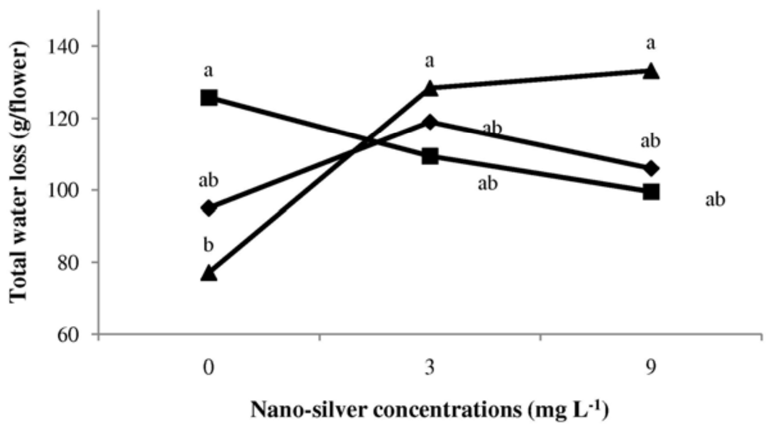

Figure 4. Interaction of nano-silver (0, 3 or 9 mg L^{-1}) and calcium sulfate concentrations (◆: 0 mM; ■: 10 mM; ▲: 20 mM) on the total water loss (g/flower) in cut gerbera flowers. The means with similar letters have no significant differences (*P*< 0.05).

In all days of measurement, cut gerberas treated with NS 3 mg L^{-1} and CS 10 mM + GA_{4+7} 20 mg L^{-1}, or CS 20 Mm + GA_{4+7} 20 mg L^{-1} had highest RFW% and with DI+ CS 10 mM (without GA) had lowest RFW% (because of tripartite interactions, the data not presented).

DISCUSSION

The pulse treatment with 3 mg NS L^{-1} and preservative solution containing 20 mM CS caused longest vase life. It was added to vase life compared to the control treatment for 7.5 (i.e. 62.5%) days (Figure 1). Gerbera is insensitive to ethylene (Liu et al. 2009a); therefore, the effect of NS to extend vase life of gerbera is not as an anti-ethylene agent,and its main role is prevention of bacterial plugging of the xylem (Liu et al. 2009a,b, Solgi et al. 2009, Chaloupka et al. 2010, Lü et al. 2010), then increasing of water uptake and calcium. According to Geraspolus and Chebli (1999) in postharvest period, calcium uptake increased stability and mechanical strength of cell wall by maintaining middle lamella as intact, and it prevented appearing the symptoms of end of vase life in gerbera [wilting, petal curling, stem bending (in this cultivar was not observed) and breaking (in this cultivar was observed partially)]. In preservative solutions with 20 mM CS without GA_{4+7} was obtained longest (4.6 days, i.e. 32.3% more than C0G20) vase life which had significant ($P \leq 0.05$) differences by solutions containing 20 mg L^{-1} GA_{4+7} without CS (Figure 2). Gibberellin can enhance

Table 1. Vase life of gerbera flowers regressed against total water uptake, total water loss.

Vase life	Linear model				
Variable	B	S.E.B	Standard *β*	*t*	Significant
Constant	9.975	3.144		3.173	0.006
Total water loss	0.062	0.028	0.486	2.224	0.041
Multiple *R*	0.486				
R^2	0.236				
Adjusted R^2	0.188				
Standard error	2.207				

ANOVA					
	Sum of squares	df	Mean square	*F*	Significant
Regression	24.098	1	24.098	4.947	0.041
Residual	77.933	16	4.871		
Total	102.031	17			

hydrolyzation of starch to glucose and fructose. Combination of these sugars results producing more sucrose and strength of cell walls. Having more sugar in tissues preserves them of early disruption and increases their longevity (Halevy & Mayak 1981). In addition, Whitman et al. (2001) determined that spraying with GA_{4+7} on *Lilium longiflorum* had a positive effect to decrease foliar chlorosis, and it increased vase life. However, in our experiment, GA_{4+7} decreased the vase life inversely, moreover, it decreased the effect of CS. The treatment of flowers with NS (3 or 9 mg L^{-1}, respectively) and 20 mM CS, by pulse treatment with DI followed with 20 mM CS caused 81.65% and 77.94% higher total water uptake (Figure 3). Sucrose acts as a food resource and maintaining of water balance, but its application in the preservative solutions without antimicrobial agents is occasioning the blockage of xylem vessels (Solgi et al. 2009). The NS pulsing prevents it and increases total water uptake. By applying Ag nanoparticles well before the penetration and colonization microbes within the plant tissues, can improve their effectiveness (Nair et al. 2010). In addition, ions in water, particularly cations, can add to flow across xylem vessels, as Liu et al. (2009a) reported NS treatments increased uptake rates in cut gerberas.

In all the treatments, total water loss was ever higher than total water uptake. In addition, these treatments increased total water loss. Generally, water availability for plant is an important and limited factor in the transpiration (Meyer et al. 1973). The greater part of transpiration is with stomata cells. Calcium ions on the contrary of potassium ions when enter to the stomata cells from epidermal cells, they cause decreasing turgescence of them and closing stomata cells. Therefore, it will decrease transpiration (Meyer et al. 1973, Mithöfer & Mazars 2002). To enhance the water uptake because of antimicrobial activity of NS affected the hydrodynamic conditions of plant and stomata mechanism. It caused increasing total volume of water in plant and in the all cells. This increased water volume transferred to the stomata cells, and increased them turgescence and transpiration (Meyer et al. 1973). Against of our results, Lü et al. (2010) showed that NS pulsing suppressed water loss in cut roses. This may be because of interaction NS and CS in our experiment.

According to the results of path analysis (Table 1), and significant effects of water loss on the vase life, water loss and the treatments that increased water loss caused the increasing the vase life. To identify the physiological basis of this effect, it is required more researches.

According to other experiments on the cut gerberas and roses (Liu et al. 2009a, Solgi et al. 2009, Lü et al. 2010) in vase period, NS treatments were ever increased RFW% compared to the control. In our experiment (Figure 4), interaction of NS (particularly 3 mg L^{-1}) and CS and GA_{4+7} were increased that up to 21 days. In addition, De Capdeville et al. (2005) reported that increasing CS concentration enhanced vase life of cut roses. This may be a reason of calcium effects to delay the senescence process by postponing cell membrane degradation (Sosa Nan 2007) and maintaining RFW%.

In conclusion, we suggest pulse treatment by NS at 3 mg L^{-1} along with continuous treatment with CS at 20 mM to improve physiological status or water relations of cut gerbera and increasing the vase life. According to significant effect of transpiration (water loss) on the vase life and is not significant the effect of GA on this factor, we do not suggest applying GA in order to decrease preserving cost.

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