

Effects of nano-silver pulsing, calcium sulfate and gibberellin on an antioxidant molecule and vase life of cut gerbera flowers

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Abbreviations: nano-silver= NS; deionized water= DI; calcium sulfate= CS; gibberellin₄₊₇ = GA; anthocyanin leakage= AL; total soluble solids= TSS.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: The aim of this study was to evaluate interactions between NS coupled with CS and GA on flavonoid, cell membrane behavior and extending the vase life of cut gerbera. Pulse treatments of flowers were conducted in NS at concentrations of 0 (DI), 3 or 9 mg/l for 24 h. Then, flowers were treated with preservative solutions containing calcium sulfate (0, 10 or 20 mM) and GA₄₊₇ (0 or 20 mg/l), plus 1.5% sucrose in all preservative solutions. Pulse treatments with 3 or 9 mg NS/l and holding in solution containing 20 mM CS compared to the control treatment (holding in the solution of sucrose following pulse treatment in DI) significantly extended vase life by 8 days. According to the antioxidant role of flavonoids, and lower amounts of flavonoid in the flowers that pre-treated with NS, therefore, it may be said that NS prevented from microbial attack.

1. Introduction

Gerberas (*Gerbera jamesonii*) are well-known flowers for the variety of their colors, and are popular in the world flower trade (Liu *et al.*, 2009 a; Solgi *et al.*, 2009). However, often the growers and florists suffer further loss from short vase life of gerberas. For example, the mean of vase life in some cultivars of *Gerbera* ('Bayadere' and 'Sunway') are reported only between 6 to 10 days when water tap is used (Shabanian *et al.*, 2018). Gerberas are ethylene insensitive, but bacterial plugging of the xylem is a main cause of early and rapid senescence in their cut flowers (Liu *et al.*, 2009 a). The decrease of water uptake and consequently the increase of the ratio between transpiration and water uptake (i.e., high value of

water balance) will be caused as a result of xylem obstruction, and will end the cut flower vase life. Nano-silver (NS) pulse and continuous treatments for cut flowers are newly used and are known as novel agents of anti-microbial (Liu *et al.*, 2009 a; Solgi *et al.*, 2009; Lü *et al.*, 2010). As a novel antiseptic, NS is used in the medical industry, silver embedded fabrics, water purification and vegetable disinfection. Due to their high surface area to volume ratio, among other unique chemical and physical properties, NS formulations provide full contact with microorganisms and are highly effective as germicides. NS particles can connect the cell membranes and penetrate into bacteria. Then, NS can disrupt the respiration and cell division and cause the cell death. NS releases silver ions (Ag^+) within bacterial cells, silver ions have bactericidal activity (Liu *et al.*, 2009 a; Solgi *et al.*, 2009; Nair *et al.*, 2010; Sharon *et al.*, 2010; Lü *et al.*, 2010; Liu *et al.*, 2012). Naghsh (2010) described the inhibited meiosis in *Aspergillus niger* due to NS activity. Alavi and Dehpour (2010) reported that the nano-silver solution is effective on greenhouse cucumber downy mildew disease. Liu *et al.* (2009 a) and Lü *et al.* (2010) observed that NS pulse treatments extended vase life of the cut gerbera and the rose flowers. Also, Solgi *et al.* (2009) reported that NS continuous treatments inhibited the growth of bacteria in the solution and xylem vessels and increased vase life of cut gerbera flowers.

Calcium increase postharvest longevity of fresh cut flowers (Gerasopoulos and 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, stem bending (Sosa Nan, 2007).

Since the level of soluble carbohydrates will be maintained by the treatment of gibberellin (GA) (Ranwala and Miller, 2000; Whitman *et al.*, 2001; Hatamzadeh *et al.*, 2010), therefore, GA can have a positive effect on the water balance. Furthermore, sucrose in the preservative solutions maintains water balance, in addition to act as a food source (Solgi *et al.*, 2009).

The objective of this research was to evaluate the interactions of calcium sulfate and gibberellin continuous treatments by NS pulse treatments on flavonoid as an antioxidant component, and vase life of cut gerberas. Flavonoids have antioxidant effects and can be effective on the vase life. Antioxidant molecules

can be efficient systems to protect cells against pathogen and water deficit-induced oxidative stress, and this prevents the senescence and cell death (Shabanian *et al.*, 2018).

2. Materials and Methods

Plant material

Cut gerbera (*Gerbera jamesonii* cv. Pink Elegance) flowers that were grown in standard hydroponic greenhouse conditions were purchased from a flower and plant growing company (Pakdasht, Tehran, Iran). Flowers were harvested by pulling the stems off in the plants when 2-3 rows of stamens of the bisexual disc florets were mature (Gerasopoulos and Chebli, 1999; Solgi *et al.*, 2009) in the morning. Stem bottom of harvested flowers was put in the flower capsule containing deionized water (DI). Flowers were packed and transported within 8 h to the laboratory. In the laboratory, stems were re-cut to a length of 45 cm into the DI to remove air emboli (Liu *et al.*, 2009 a; Solgi *et al.*, 2009). Flowers were re-cut 2-3 times, when it was necessary.

The flowers were placed in a controlled environment room at $20\pm 2^\circ\text{C}$ with $60\pm 10\%$ R.H. and $12\ \mu\text{mol/m.s.}$ light intensity (cool white fluorescent lamps; 12 h/day).

Experimental design and treatments

Solutions of pulse treatment were prepared in two concentrations of NS (3 or 9 mg/ l), and DI was used as a control treatment. Flowers were treated for 24 h with the two concentrations of NS (Nanonasb-Pars Company, Iran) or DI. Each pulse treatment contained 18 flowers. Following pulse treatment, the flowers were individually kept into 1000 ml glass vases containing 500 ml of fresh solutions (as continuous treatment) that were prepared at second day of the experiment and were not renewed. In the continuous treatments, three concentrations (0, 10 or 20 mM) of calcium sulfate, CS, ($\text{CaSO}_4\cdot 2\text{H}_2\text{O}$, Merck Company), and two concentrations (0 or 20 mg/ l) of GA_{4+7} (Serva Company, USA) were used. In the all continuous treatments, sucrose 1.5% was used.

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 three-way 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. Correlation coefficients between vase life and cell conditions and flavonoid were conducted by SPSS (version 11.5). Regression analysis (path analysis) was taken to determine the major factors that affect vase life (dependent variable). The independent explanatory variables were anthocyanin leakage, tissue pH, TSS and flavonoid. The software used for path analysis was SPSS/PC+ "Stepwise" (version 11.5).

Vase life

Vase life was recorded from harvest time by the time the flowers showed symptoms of petal wilting or curling, stem bending ($\geq 90^\circ$) or breaking, therefore, the flowers were visited daily.

Total flavonoid assay

In termination of the vase life, to extract total flavonoid, 20 ml of acidic methanol (1% HCl) was added to 0.2 g fresh weight of petals, and the mixture was stirred for 48 h in the dark. The extract was used to measure total flavonoid content immediately (Chang *et al.*, 2002). Total flavonoid content was measured by aluminum chloride colorimetric assay (Chang *et al.*, 2002; Kumar *et al.*, 2008). An amount of 200 μ l of plant extract was added to 600 μ l of methanol, 40 μ l AlCl_3 (10%), 40 μ l of potassium acetate (1 M) and was made to 2000 μ l by distilled water. The solution was vigorously mixed and after keeping at room temperature in the dark for 30 min, the absorbance was measured against reagent blank at 510 nm with a spectrophotometer (T80+ UV/VIS Spectrometer, PG Instruments Ltd). The calibration curve of standard solutions of catechin (5-40 μ g/ml of 1% HCl in methanol) was drawn ($y = 0.0003x + 0.1654$, $R^2 = 0.9989$). Total flavonoid content of flower was expressed as mg catechin equivalent per 100 g of fresh weight.

Anthocyanin leakage

To evaluate the effects of treatments on the cell membrane structure, anthocyanin leakage was measured to observe the stability of plasma membrane. At tenth day of vase life, 0.5 g of petals was sliced to pieces of 1×1 cm, these pieces were washed in DI water two times and within a period of 2 h. Then 10 ml of DI water was added to samples. After 12 h in 25°C, the absorbance was recorded with a spectrophotometer (T80+ UV/VIS Spectrometer, PG Instruments Ltd) at 525 nm (Poovaiah, 1979).

Tissue pH measurement

Tissue pH was measured according to the method

of Hill (1999) to consider the treatment influences on the conditions of cell reactions. In termination of vase life, 2 g of petals was crushed in liquid N_2 , and then was placed at -80°C for 48 h. The frozen tissues were removed from -80°C , thawed at 20°C , then were frozen in liquid N_2 again and placed at -80°C for a further 36 h. After thawing at 20°C again, 25 ml distilled water was added to tissues in the test tube, then were frozen at -20°C for 24 h. The pH of filtered fluid was recorded after thawing with a pH meter.

Total soluble solid

Total soluble solid (TSS) of petal juice was measured with a refractometer (CET1, Belgium) as °Brix (Rooin *et al.*, 2009).

3. Results and Discussion

Interactions between NS and CS significantly extended the vase life (Fig. 1). NS pulse treatments and then holding in preservative solutions (10 or 20 mM CS) extended the vase life compared to DI pulsing and preservative solution containing sucrose (control treatment) (Fig. 1). However, no significant ($p < 0.05$) difference was found among various concentrations of NS and CS to extend the vase life. Also, interactions between CS and GA_{4+7} had significant effect on the extension of the vase life ($p < 0.05$) (Fig. 2). The longest vase life was found in the pulse treatment with 3 mg NS/l and preservative solution containing 20 mM CS. It was 7.5 days (i.e., 62.5%) added to vase life compared to the control (Fig. 1). Gerbera is insensitive to ethylene (Liu *et al.*, 2009 a); there-

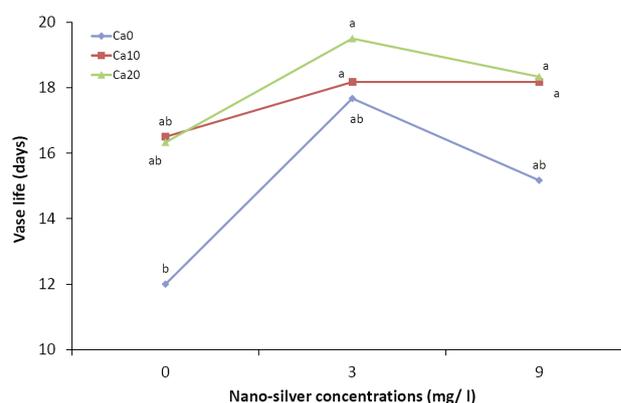


Fig. 1 - Interactions between nano-silver (0, 3 or 9 mg/l) and calcium sulfate concentrations (\blacklozenge = 0 mM; \blacksquare = 10 mM; \blacktriangle = 20 mM) on the vase life (days) in cut gerbera flowers. The means with same letters have no significant differences ($MS_{ANOVA} = 6.27$; Type 1 Error, $HSD_{0.05}$, $N=3$).

fore, NS effects for extending the vase life of gerbera are not related to anti-ethylene effects of NS. Therefore, it must be explained that the basic role of NS is to prevent from bacterial plugging of the xylem (Liu *et al.*, 2009 a, b; Solgi *et al.*, 2009; Chaloupka *et al.*, 2010; Lü *et al.*, 2010); then increasing water uptake and calcium. According to Gerasopoulos and Chebli (1999), post-calcium uptake prevents appearing the symptoms of the 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)]. The longest vase life was obtained in preservative solutions containing 10 mM CS without GA₄₊₇ [approximately 19 days, i.e., 32.3% more than treatment with 20 mg/l GA, without CS, which had significant (p<0.05) differences with solutions containing 20 mg L⁻¹ GA₄₊₇ without CS] (Fig. 2). Gibberellin can enhance hydrolization of starch to glucose, and during enzymatic process sucrose will be produced. More production of sucrose causes strength of cell walls. Having more sugar in tissues preserves them of early disruption and increases their longevity (Halevy and Mayak, 1981). Also, Whitman *et al.* (2001) determined that GA₄₊₇ sprayed

to *Lilium longiflorum* had a positive effect to decrease foliar chlorosis and increased vase life contrary to our results; moreover, it decreased the effect of CS.

There were significant interactions (p<0.05) between GA, CS and NS on total flavonoid at the end of vase life (Table 1). The highest flavonoid content

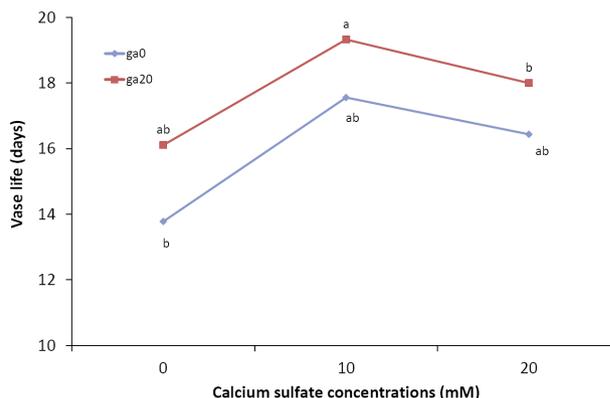


Fig. 2 - Interactions between calcium sulfate (0, 10 or 20 mM) and GA₄₊₇ concentrations (◆ = 0 mg GA₄₊₇/l; ■ = 20 mg GA₄₊₇/l) on the vase life (days) in cut gerbera flowers. The means with same letters have no significant differences (MS_{ANOVA} = 1.41; Type 1 Error, HSD_{0.05}), N=3.

Table 1 - The effect of nano-silver pulsing and continuous treatments by calcium sulfate and gibberellin on the biochemistry factors of cut gerbera flowers

Pulse treatment NS (mg/l)	Treatments		Flavonoid (mg equivalent catechin per 100 g F.W.)	Anthocyanin leakage (Absorbance in 525 nm)	Tissue pH	TSS (° Brix)
	Continuous treatment CS ² (mM)	GA (mg/l)				
0	0	0	3933.3 bcd	0.165 a	4.98 abcd	11.2 a
		20	2350.0 d	0.160 abc	5.15 a	10.8 ab
	10	0	4516.7 abcd	0.161 abc	4.94 bcd	11.1 a
		20	3877.8 bcd	0.161 abc	4.98 abcd	11.3 a
	20	0	8183.3 ab	0.160 abc	4.91 bcd	11.2 a
		20	8933.3 a	0.157 bc	4.94 bcd	9.9 ab
3	0	0	5711.1 abcd	0.159 abc	4.98 abcd	9.4 ab
		20	2988.9 cd	0.163 ab	5.01 abc	10.0 ab
	10	0	5655.6 abcd	0.155 c	4.88 bcd	9.0 ab
		20	3433.3 bcd	0.157 bc	4.83 cd	8.9 ab
	20	0	7544.4 abc	0.156 bc	4.86 cd	8.8 ab
		20	5377.8 abcd	0.155 c	4.83 cd	7.9 b
9	0	0	2933.3 cd	0.156 bc	4.96 abcd	9.8 ab
		20	3100.0 cd	0.157 bc	5.05 ab	10.4 ab
	10	0	2766.7 cd	0.155 c	4.85 cd	8.3 ab
		20	5322.2 abcd	0.156 bc	4.89 bcd	8.5 ab
	20	0	6488.9 abcd	0.156 bc	4.82 d	9.5 ab
		20	3711.1 bcd	0.157 bc	4.86 cd	8.9 ab
ANOVA (Mean of Square)						
NS			815234.8 NS	0.0001 **	0.04 **	18.97 **
CS			50446502.1 **	0.0001 **	0.12 **	4.47 *
GA			12438400.2 *	0.00000002 NS	0.02 *	0.74 NS
NS x CS			6853137.9 y	0.00002 *	0.0008 y	1.56 y
NS x GA			696546.46 y	0.00002 *	0.01 y	0.47 y
CS x GA			2485082.3 y	0.000003 y	0.01 y	1.52 y
NS x CS x GA			5213477.41 y	0.00001 y	0.0009 y	0.1 y

The means with different letters are significant (HSD_{0.05}). N= 3. ** = significant at p<0.01; * = significant at p<0.05; NS = not significant. y= Type I Error.

was measured in the petals of flowers that were treated with DI and kept in the solution containing 20 mM CS and 20 mg/l GA. Whereas treated flowers with DI and kept in the solution containing 20 mg/l GA showed the lowest total flavonoid. The antioxidant role of flavonoids was revealed for the flowers that were not pulsed by NS, because, the microbial attack might be a signal to synthesize the flavonoids (Khawiwora *et al.*, 2010). When calcium (Meyer *et al.*, 1973) and gibberellin (Ranwala and Miller, 2000; Hatamzadeh *et al.*, 2010) were made available, they activated the reducing of nitrate to produce phenylalanin, and to form simple carbohydrates, respectively. Therefore, the pathway of flavonoid synthesis was completed and total flavonoid was increased. Phenylalanine transforms into 4-coumaroyl-CoA in the phenylpropanoid pathway, and finally enters the flavonoid synthesis pathway (Falcone Ferreyra *et al.*, 2012).

The most significant interactions ($p < 0.05$) were observed between GA, CS and NS on the anthocyanin leakage. In the flowers which were not treated with NS and/or CS, anthocyanin leakage was highest (Table 1). The stability of cell membrane will have been decreased by factors as senescence, microbial (attacking by micro-organism) or no microbial (deficit of calcium) diseases. The measurement of anthocyanin leakage at half of vase life could be a gauge to evaluate the stability of cell membrane. The accumulation of calcium in middle lamella of cell wall increases the stability of cell membrane and decreases anthocyanin leakage (Nikbakht *et al.*, 2008). NS prevents microbial attack and decreases senescence and keeps stability (Liu *et al.*, 2009 a; Solgi *et al.*, 2009; Lü *et al.*, 2010).

The most tissue pH was recorded at pre-treatment with DI and keeping in solution containing 20 mg GA/l, and the least tissue pH was at pulsing with 9

mg NS/l and keeping in calcium sulfate solution (20 Mm). Generally, significant differences were observed between treatments ($p < 0.05$) (Table 1). Schmitzer *et al.* (2010) explained that increasing the cell sap pH causes the developing flowers from the bud to senescence stage.

The most TSS was measured in the flowers of control treatment, and the least TSS was recorded in the flowers which were pulsed with 3 mg NS/l and kept in solution containing 20 mM CS and 20 mg GA/l. Significant differences ($p < 0.05$) were observed between two mentioned treatments. However, no one has the significant differences with other treatments. Gebremedhin *et al.* (2013) interpreted that TSS will be increased by more water uptaking to provide the required substrate for respiration.

The analysis of correlation coefficients (Table 2) shows the negative significant correlation ($p \leq 0.01$) between vase life and anthocyanin leakage and TSS. Furthermore, there is positive significant correlation between anthocyanin leakage and TSS (Table 2). For the last parameter, the coefficient of multiple determinations (R^2) was 0.479 in linear model for the vase life (Table 3). This coefficient gives the proportion of the total variation in the dependent variable (vase

Table 2 - Correlation coefficients between vase life (days), anthocyanin leakage (AL), tissue pH, total soluble solids (TSS) and flavonoid (mg equivalent catechin per 100 g F.W.), N=3

Parameters	Vase life	AL	Tissue pH	TSS	Flavonoid
Vase life	1				
AL	-0.656 **	1			
Tissue pH	-0.353	0.412	1		
TSS	-0.692 **	0.757 **	0.375	1	
Flavonoid	-0.338	0.253	-0.373	0.206	1

** Significant in $p < 0.01$ (two-tailed correlations), N=18.

Table 3 - Vase life of gerbera flowers regressed (stepwise regression) against anthocyanin leakage, tissue pH, TSS and flavonoid

Vase life		Linear model			
Variable	B	SE B	Standard β	t	Significance
Constant	32.265	4.04		7.987	0
TSS	-1.586	0.414	-0.692	-3.832	0.001
Multiple R	0.692				
R^2	0.479				
Adjusted R^2	0.446				
Standard error	1.82				
ANOVA					
	Sum of squares	df	Mean squares	F	Significance
Regression	48.834	1	48.834	14.688	0.001
Residual	53.197	16	3.325		
Total	102.031	17			

life) explained by the predictors included in the model. Thus, from among four independent variables, total soluble solids explained 47.9% of the observed total variation in the vase life, and other independent variable (anthocyanin leakage, tissue pH and flavonoid) had a lesser role in the vase life. Furthermore, the test statistic in linear model showed that coefficient of TSS negatively and significantly ($p \leq 0.01$) influenced vase life (Table 3, Fig 3). Therefore, the factors that cause the increased TSS and anthocyanin leakage lead to decrease vase life. This is also confirmed by other researchers (Gebremedhin *et al.*, 2013).

According to the results, we recommend pulse treatment with 3 mg NS/l and then, continuous treatment with 20 mM CS for increasing the vase life of cut gerbera by 8 days.

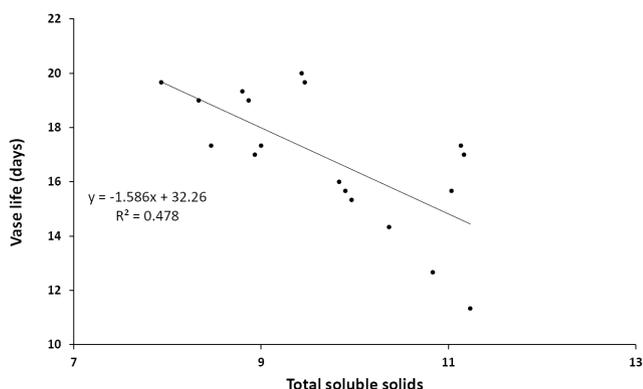


Fig. 3 - Relationship between vase life and total soluble solids, N=3.

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