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Postharvest Foliar Application of Gibberellic acid and Calcium chloride Improved vase life and Water Balance of cut rose Flower cv. Velvet

Rahim Naghshiband Hassani* and Fatemeh Alimirzaii**

**Assistant Professor, Department of Horticulture Sciences,*
Faculty of Agriculture, University of Tabriz. Iran
***Graduated M.Sc. Student, Dept. of Horticulture, Faculty of Agriculture, University of Tabriz.*

(Corresponding author: Rahim Naghshiband Hassani, rahnaghsh@yahoo.com)
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ABSTRACT: The aim of this study was the better assessment of the relationship between postharvest foliar application of gibberellic acid and calcium chloride as anti-senescence agents on improving some qualitative and physiological attributes of cut rose flower cv. Velvet. Hence, an experiment was conducted as the foliar treatment of cut stems with gibberllic acid (GA3) at 0, 0.5, 1 and 1.5 mM, and calcium chloride (Ca) at 0, 5, 10 and 15 mM, as the cut stems were held in sucrose solution at 0 and, 2 % with 250 mg.L^{-1} of 8- HQS as an antimicrobial agent for all holding treatments. The study was performed as a factorial experiment based on a completely randomized design (CRD) with three replication for each combination treatment. Different concentrations of GA3 and $CaCl_2$ increased the vase life of "Velvet" cut rose. The longest vase life (17.8 days) was observed in the combination of GA3 1mM with calcium chloride 15 mM concentrations. The effect of GA3 postharvest foliar application in increasing fresh weight of cut stem and improving of solution uptake of cut rose stem extended by increasing in calcium chloride concentration. The effect of calcium chloride alone in flower opening of cut rose stem was significantly lower than GA3 treatment combinations. In conclusion, the concentrations of 1.5 mM of GA3 with calcium chloride 15mM was the most effective postharvest foliar treatment in extending vase life by improving water balance of cut rose stem cv velvet.

Keywords: Plant growth regulators, Calcium, Water uptake, Vase-life, Cut rose

INTRODUCTION

Senescence of cut flowers is under hormonal control and related to the changes in the carbohydrate status of the petals (Halvey and Mayak, 1981). The GA3 is considered to be a senescence- delaying plant growth regulator (Arteca, 1996). Pulsing alstromeria flowers for 24 h with a 0.01 mmol GA3 solution increased longevity of cut flowers (Jordi *et al*., 1995). Sabehat and Zieslin (1995) also noted that GA3 treatment increased the vase life of roses. Hunter *et al*., (2004) found that treatment with GA3 repressed accumulation of the seven senescence associated transcripts in daffodil. It has recently been shown that treatment of flower with cytokinins and gibberellins can delay the senescence of cut flowers. Results of Ganelevin and Zieslin (2002) showed that it is possible that sepals are as a source of gibberellic acid during flower bud development. Removing sepals, reduces fresh and dry weight, with the buds and peduncle length. Gibberellic acid changes critical rate of ethylene that increase vase life (Saks and Staden, 1993).

Singh *et al.,* (2008) demonstrated that the vase solution treatment combinations of GA3 and benzyladenine with sucrose significantly increased the vase life of cut spikes of gladiolus as compared to the sucrose alone treatment or the control. However, the results obtained have been variable. Boose and van Staden (1989) demonstrated that the efficiency of these compounds depends on the mode of application as well as the type and concentration of a cytokinin used. The aim of the present work was to determine the effect of benzyladenine and gibberellic acid at different concentrations, used in a pulse treatment solution, on the longevity of rose 'Red One'.

Calcium may be involved in control of membrane stability and senescence of plant cells (Torre *et al*., 1999; Rubinstein, 2000). Alterations of the intercellular and/or cytosolic concentrations of calcium may trigger either catabolism or remodeling and the turnover process of the cell membrane components. Calcium content in the tissue affects many processes during plant growth, at all stage of development (Ferguson and Drobak, 1988).

The use of calcium in vase solutions increases water flow through the stems by association with pectin in the xylem cell walls (Van Ieperen and Van Gelder, 2006). Data from several studies with different cut flower species, *Rosa hybrida* (Bhattacharjee and Palanikumar, 2002; De Capdeville *et al*., 2005; Michalczuk *et al*, 1989; Torre *et al*, 1999), *Dianthus caryophyllus* (Mayak *et al*, 1978), *Gerbera jamesonii* (Gerasopoulos and Chebli, 1999) and *Gladiolus* (Pruthi *et al*, 2001), indicate that calcium may increase postharvest longevity of cut flowers. Other studies have shown that supplemental calcium applied as calcium nitrate [$Ca(NO_3)_2$], calcium chloride ($CaCl_2$), and calcium sulfate ($CaSO_4$) (Bhattacharjee and Palanikumar, 2002; De Capdeville *et al*, 2005; Michalczuk *et al*, 1989; Torre *et al*, 1999) may decrease the rate of senescence or increase postproduction longevity.

Considering crucial role of calcium and giberellic acid in maintaining plant cell membrane and cell wall integrity, it is important to investigate their role in regulating the flower senescence in rose flower. Therefore, an experiment was conducted to understanding the physiological role of gibberellic acid and calcium in relation to flower senescence in cut rose flower.

MATERIALS AND METHODS

A. Plant Material, Experimental Design and Treatments

Cut rose flowers cv. Velvet (*Rosa hybrida*) was prepared from Mahan commercial greenhouse in Tabriz, Iran. This experiment was performed as a factorial experiment based on completely randomized design (CRD) consisting of 16 treatments and three replications with 4 cut stems in each treatment combination. As soon as the flowers arrived to the lab, their thorn and lower leaves of the cut stem was removed gently. Prior to treatment, rose stems were trimmed to a length of 50 cm and then they foliar treated with gibberellic acid (GA3) solution at four levels (0, 0.5, 1 and 1.5 mM) and calcium chloride (Ca) at 0, 5, 10 and 15 mM, as the cut stems were held in sucrose solution at 0 and, 2 % with 250 mg/l of the 8-hydroxyquinoline sulfate (8-HQS). Cut flowers were kept at room temperature (20 ±2°C), relative humidity 60±5% and the light intensity of 12 $\mu mol/m^{-2}.s^{-1}$ of cool white fluorescent lamps with 12 hours of light until the end of vase life.

B. Measurements

Vase life. During the vase-life period, the visual quality of cut flowering stems was inspected daily. In our study, vase-life was defined as the period from the time of cutting to the time when 50% of floret petals wilted or abscised or floret necks bent as described by Liao *et al*., (2000).

C. Solution uptake, relative fresh weight and leaf chlorophyll index

The cut flowers fresh weight and the solution uptake rate were measured daily. The weight of vases with and without cut flowers was recorded daily. Mean daily solution uptake (g $stem^{-1}$ day^{-1}) was computed using the formula (St_{-1}-St), where St is the weight of vase solution (g) at t=day 1, 2, 3,…, and n. Relative fresh weight (RFW) of stems was computed using the formula RFW (%)=(Wt/W0)×100, where, Wt is the weight of stem (g) at t=day 0, 1, 2…, and n, and W0 is the weight of the same stem (g) at t=day 0 (He *et al*., , 2006). Chlorophyll Index was measured by a SPAD-502 (Minolta Co., Japan). All readings were carried out between the tip and the base of fully expanded leaves in each sample.

D. Flower diameter, petal dry weight and water content

Flower diameter was measured as an index for petals expanding rate. The outer diameter of opened flowers was measured by a Vernier Caliper (mm). Fresh weight of petals was recorded and petal dry weight was recorded after drying at 105 °C for 48 h in an electrical oven until constant weight was obtained. Petal water content was determined as the percentage of total petal weight [(FW - DW)/FW×100] by weighing samples of all petals from a single flower.

E. Statistical Analysis

The recorded data were subjected to analysis of variance (one-way ANOVA) using the general linear model program of SPSS software (SPSS Ver. 16). Means were compared by the least significant difference (LSD) test at the 0.05 probability level.

RESULTS AND DISCUSSION

A. Vase life

Results showed that following GA3 and $CaCl_2$ postharvest floiar application vase life was extended significantly ($P<0.01$). Different concentrations of GA3 and $CaCl_2$ increased the vase life of "Velvet" cut rose. The longest vase life (17.8 days) was observed in the combination of GA3 1mM with calcium chloride 15 mM concentrations. Minimum vase life (7.2 days) belonged to the control stems (Table 1). The correlations between vase life and some physiological characters of cut stem was presented in Table 3 which has been showed that there was a negative correlation between vase life and flower diameter and petal dry weight.

It has been reported that GA3 delays wilting and senescence as associated proteolysis (Eason, 2002). In an experiment on 5 rose cultivars which was performed by Goszczynska *et al*., (1990), vase life of cv. Mercedes increased significantly as in detached petals by gibberellic acid treatment. Sairam *et al*. (2011) reported that the vase life of Gladiolus flowers was increased by calcium chloride holding solution. Salts of calcium influence the vase-life of flowers through their mode of action, which includes their role in signaling, plant metabolism and maintenance of cell wall stability (Agarwal *et al*., 2005).

Table 1: Interaction effect of gibberellic acid (GA3) and calcium chloride postharvest foliar application on vase life (days) of cut rose cv. Velvet.

Treatments	Calcium(mM)			
GA_3 (mM)	0	5	10	15
0	7.2 f	9.5 e	11.2 d	11d
0.5	12.8 c	13.6 c	15 a	16.5b
1	13.2 c	15.4 b	15.7b	17.8a
1.5	15.5b	16b	16.2b	17.5a
$LSD_{0.05}$= 1.6 (n= 3)				

Each value represents a mean of three replicates. Means followed by the same letters were not significantly different at 5% level of significance.

B. Solution uptake, relative fresh weight and leaf chlorophyll index

Combination treatment of GA3 and calcium chloride was significantly (P<0.001) effective in extending cut rose stem relative fresh weight at all concentrations (0.5, 1 and 1.5 mM) during vase life period. The highest value (105.7%) of relative fresh weight was observed in GA3, 0.5mM and calcium 15 mM compared with other treatments. These results showed that the effect of GA3 postharvest application in increasing fresh weight of cut stem extended by increasing in calcium chloride concentration (Table 2). Solution uptake of cut stems, improved significantly (P<0.01) by gibberellic acid treatment and its effect was extended by calcium chloride concentration in cut stem solution uptake (Table 2).

Table 2: Interaction effect of GA3 and calcium chloride postharvest foliar application on cut rose stem cv. Velvet water balance during vase life.

GA_3 (mM)	Calcium chloride (mM)	Relative fresh weight (%)	Solution Uptake ($g.day^{-1}.stem^{-1}$)	Leaf chlorophyll Index	Flower diameter (mm)	Petal dry weight (g)	Petal water content (%)
0	0	92.4d	1.5d	49.2d	53.5b	0.12d	62.3d
	5	91.6d	1.7d	50.4d	46.7c	0.11d	63.2d
	10	94.8cd	1.9d	52.3c	42.5d	0.15c	65.4c
	15	96.7c	2.2c	52.6c	40.2d	0.13d	66.7c
0.5	0	96.5c	1.8d	51.2c	45.4d	0.15c	65.1c
	5	98.5c	2.1c	54.7b	43.7d	0.17c	66.7c
	10	102.4b	2.8c	57.2c	45.5c	0.19c	68.4bc
	15	105.7a	3.2b	58.4a	46.2c	0.2c	69.2bc
1	0	96.7c	2.3c	51.7c	45.6c	0.2c	69.5bc
	5	99.5bc	2.9c	53.5b	48.9c	0.25b	70.5b
	10	105.6a	3.7b	54.6b	52.3b	0.28b	72.3b
	15	104.5a	4.2a	58.7a	53.6b	0.29ab	72.4b
1.5	0	97.5c	2.2c	52.4c	47.3c	0.30a	73.2b
	5	101.7b	3.1b	56.7b	50.2b	0.29a	75.6b
	10	105.2a	3.6b	57.9a	52.4b	0.36a	80.4a
	15	104.3a	4.7a	59.2a	54.7a	0.32a	83.7a
$LSD_{0.05}$ (n= 3)		2.36	1.02	2.07	1.98	ns	2.68

Each value represents a mean of three replicates. Means with the same letter were not significantly different at 5% level of significance (P<0.05).

The maximum amount of solution uptake (4.7 g $stem^{-1}$ day^{-1}) was observed at the combination treatment of GA3 1.5mM and calcium 15mM (Table 2). Leaf chlorophyll index of cut rose stem was significantly ($P<0.05$) increased by GA3 and calcium chloride postharvest foliar application (Table 2). The combination treatment of GA3 at 1.5 mM with Ca at 15 mM showed the maximum value (59.2) of chlorophyll index. The minimum amount (49.2) was related to control (Table 2). Pearson's correlation coefficient results showed that the highest and lowest positive correlation coefficients (0.757 and 0.347) were recorded between stem relative fresh weight and petal water content and leaf chlorophyll index, respectively (Table 3). The same trend was observed for vase life with stem relative fresh weight and solution uptake. There was negative correlation between stem relative fresh weight and solution uptake with petal dry weight. Similar results have been reported in gerbera cut flowers with GA3 treatment (Emongor, 2004 and Danaee *et al*., 2011). The GA3 along with sucrose has been suggested to induce water uptake in cut flowers of gerbera (Emongor, 2004). In fact gibberellic acid caused negative osmotic potential cell and increase water uptake by hydrolysis of starch and sucrose (Goszczynska and *et al*, 1990). Singh and *et al* (2008) reported that 50-500 mg/l gibberellic acid spray over cut roses increases water uptake. Water shortages caused when the amount of transpiration is more than water uptake (Nowak and Rudnicki, 1990). Also gibberellins continue to modulate growth of flowers after harvest as evidenced by increased fresh weight of cut roses upon application of GA3 (Sabehat and Zieslin, 1995). GA significantly protects the chlorophyll in plants and prevents leaf yellowing during postharvest period, in some plants such as lily, Alstroemeria which can be stored for a long time and caused to increase the vase life (Lukaszewska, 1995). In Easter lily leaves, the senescence delaying effect of GA3 was associated with depression of the respiration rate (Han, 1995). Saks and van Staden, (1993) reported that GA3 treatment reduced levels of ACC and ethylene production. This hormone prevents the degradation of chlorophyll in plants (Ichimura and Goto, 2000), this may be due to decrease in pH cell sap and to prevent of degradation chlorophyll that protects chlorophyll (Skutink, 2001). Gibberellic acid decrease chlorophyll degradation and loss during the senescence process, because of its role strengthening in the membrane of chloroplasts.

Table 3: Pearson's correlation coefficients between some physiological parameters and vase-life of cut rose cv. Velvet as affected by GA3 and calcium chloride postharvest foliar application.

Characters	Vase life	Relative fresh weight (RFW)	Solution uptake(SU)	Leaf chlorophyll index(LCI)	Flower diameter (FD)	Petal dry weight (PDW)	Petal water content (PWC)
Vase life	1.000	-	-	-	-	-	-
Relative fresh weight	0.542^{ns}	1.000	-	-	-	-	-
Solution uptake	0.731*	0.714*	1.000	-	-	-	-
Leaf chlorophyll index	0.326^{ns}	0.347^{ns}	0.438^{ns}	1.000	-	-	-
Flower diameter	-0.478^{ns}	0.543^{ns}	0.751*	0.252^{ns}	1.000	-	-
Petal dry weight	-0.282^{ns}	-0.680*	-0.420^{ns}	0.425^{ns}	-0.384^{ns}	1.000	-
Petal water content	0.785*	0.757*	0.855**	0.373^{ns}	0.527^{ns}	-0.739*	1.000

C. Flower diameter, flower petal dry weight and water content

Our results showed that GA3 postharvest foliar treatment had a significant effect ($P<0.01$) on the amount of flower opening as it is increased. The effect of calcium chloride alone in flower opening of cut rose stem was significantly lower than GA3 treatment combinations. The maximum flower bud opening (56.7mm) was observed at GA3 1.5 mM with calcium chloride 15 mM treatment and its minimum rate was belong to control (36.5 mm) (Table 2). There was a significant difference ($P<0.05$) among flower petal dry weight values for both treatments. These results showed that with increasing in GA3 concentrations the value of petal dry weight was higher such as higher amounts of flower petal water content was observed in concentration of 1.5 mM of GA3 with 15mM concentration of calcium chloride treatment compared with control.

Similar results was observed for flower petal content as the maximum rate of flower petal content (83.7%) was observed in GA3 1.5 mM with calcium chloride 15 mM treatment (Table 2). The positive and significant correlation coefficient of flower diameter was observed with solution uptake content of cut rose flower (0.751). Petal dry weight showed negative significant correlation with relative fresh weight of cut stem (-0.680) and petal water content showed a positive significant correlation with solution uptake (0.855) among other characteristics (Table 3). There was a significant positive correlation between vase life and petal water content (0.785) and a significant and negative correlation was observed between petal water content and petal dry weight (-0.739). In conclusion, our results showed that the mixing application of GA3 and calcium chloride postharvest foliar application had a significant effect in regulation of water balance of cut stem as keeping water content in flower petals and declining the time of flower wilting during vase life of cut rose flower cv. Velvet. Skutnik *et al*., (2001) showed that a pulse treatment with GA3 greatly improved the postharvest performance of *Zantedeschia aethiopica* leaves and dramatically reduced the normal increase in pH and conductivity of the cell sap. The role of GA in petal growth has been demonstrated in many plants and seems to be a general phenomenon (Pharis and King 1985). Gibberellin treatments increased the size of flowers. The possibility is discussed that GA, which is exogenously supplied, enhances flower dimensions and keep flower pigmentation by drawing photosynthates to the flower as a consequence of intensification of the sink (Zieslin, *et al*., 1974). Calcium (Ca^{2+}) plays a fundamental role in plant membrane stability, cell wall stabilization, and cell integrity (Hirschi, 2004). Reduced Ca^{2+} levels in edible plant tissues negatively impacts total yield. Plant tissues low in Ca^{2+} are more susceptible than tissues with normal Ca^{2+} levels to some parasitic diseases during storage (Capdaville *et al*. 2003).

CONCLUSION

In conclusion, the effect of GA3 and calcium in extending vase life of cut rose stem was synergistically, however the concentrations of 1.5 mM of GA3 with calcium chloride 15mM was the most effective postharvest foliar treatment in extending vase life by improving water balance of cut rose stem cv velvet.

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