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## The Interrelationship between Calcium and Salicylic Acid Pulsing on Vase Life and Flower Opening of Cut Rose Flower

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ABSTRACT: This study examined the effect of pulsing salicylic acid and calcium chloride and then sucrose holding of cut rose flower cv. Velvet, on its vase life and water balance. Flowers were placed in the pulsing solution containing salicylic acid (0, 1 and 2mM), calcium chloride (0, 2.5 and 5mM) and their combinations for 24 hours. All cut stems then transferred to the holding solution with sucrose (0 and 2%) and 8-HOS (250mg.L<sup>-1</sup>) for all treatment combinations. The most effective treatment in increasing vase life (22 days) observed in the combination treatment of salicylic acid at concentration of 2mM with calcium chloride treatment of 5mM. Water uptake increased significantly by salicylic acid 2mM and sucrose 2% holding as it reached to the highest level (4.5 g. stem. day) in comparison with control. The relative fresh weight of cut stems increased significantly by salicylic acid pulsing 2mM alone and calcium chloride pulsing 5mM alone with sucrose 2% holding. The highest value of petal relative fresh weight related to calcium pulsing at 5mM alone and the concentration of 2 mM salicylic acid pulsing alone during vase life.

**Key words:** Cut rose flower, anti-senescence agents, water balance, carbohydrate supply.

### **INTRODUCTION**

Ageing processes of cut flowers during postharvest period accelerate. To delay their senescence processes and subsequently increase their vase life, postharvest treatments needed (Halvey & Mayak, 1981). The most common and visibly apparent senescence symptom in cut rose flowers is loss of cell turgor, resulting in wilting and death. Cell membranes are responsible for the regulation of the content of nutrient ions and other metabolites inside the cell by selective transport in and out of the cell, for the preservation of compartments in the cell and for water retention (Rubinstein, 2000). In fresh cut roses, experimental evidence has suggested that there is a steady decline in water uptake combined with continuous water transport and water loss (Halevy & Mayak, 1981). This relationship increases the water deficit and results in decreased water potential of the flower (Eze et al, 1986). Water potential is maintained by the water content inside the cell and the solute concentration of this intracellular water (Halevy & Mayak, 1981). It has been proposed that cell membrane deterioration, which results in solute leakage out of the cell, could contribute negatively to the components of water balance inside and outside the cell (Borochov & Woodson, 1989; Torre et al, 1999).

Research in postharvest physiology suggests that 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 & 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 & Van Gelder, 2006). Data from several studies with different cut flower species, Rosa hybrida (Bhattacharjee & Palanikumar, 2002; De Capdeville et al, 2005; Michalczuk et al, 1989; Torre et al, 1999), Dianthus caryophyllus (Mayak et al, 1978), Gerbera jamesonii (Gerasopoulos & 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<sub>4</sub>) (Bhattacharjee & 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.

Salicylic acid (SA) is an endogenous growth regulator with phenolic nature, which participates in regulation of several physiological processes in plants, such as stomatal closure, ion uptake, inhibition of ethylene biosynthesis and transpiration (Khan et al, 2003). It has been shown that postharvest treatment of various cut flowers with SA could improve their vase life (Jalili Marandi et al, 2011; Kazemi et al, 2011; Mansouri, 2012) and also it has been previously reported that salicylic acid in the vase solution promotes cut rose vase life by both delaying senescence and improving water balance (Alaey et al, 2011). Treatments that improve water balance are expected to mitigate the negative effect of prior water stress on vase life. Antitranspirant compounds, such as acetylsalicylic acid (Miura and Tada, 2014) that decrease water loss owing to stomatal closure, are expected to promote vase life of cut flowers. Salicylic acid treatment has the potential for maintenance of cut flowers quality and extension of vase life. However, there is no information on the interrelationship between salicylic acid and calcium as antisenescence agents alone and their combination treatment with carbohydrate supply in vase solution on vase life and water relations of cut rose flower. Therefore, the objective of this study was to investigate and compare the effect of pulsing salicylic acid and calcium and sucrose holding on keeping quality and vase life of cut rose flower cv. Velvet.

#### MATERIALS AND METHODS

#### A. Plant material and treatments

Cut rose flower cv. 'Velvet' was obtained from a commercial greenhouse and transported with appropriate cover (in plastic packages) immediately to the laboratory in January 2014. Cut stems were re-cut under tap water to have uniform length of 50 cm. This study was performed as a factorial experiment based on a completely randomized design with three replication for each treatment combination. Flowers were then placed in the glass vials 500 ml pulsing solution containing salicylic acid (0, 1 and 2mM), calcium chloride (0, 2.5 and 5 mM) and their combinations. The flowers were kept at  $18 \pm 2^{\circ}$ C under a 10:14h light/dark cycle (15  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>) and 60 ± 5% RH for 24 hours. After pulsing treatment all treatments transfered to the holding solution with sucrose (0 and 2%), and 8-HQS (250mg.L<sup>-1</sup>) for all treatment combinations and were kept in the condition described above for the rest of the experiment.

**Evaluation.** Vase life. Vase life was assessed as the number days to wilting of flowers. The flowers were checked once a day for signs of deterioration. In our study, vase-life was defined as the period from the time

of cutting to the time when 50% of flower petals wilted or abscised or flower necks bent (Liao *et al*, 2000).

# Solution uptake, stem relative fresh weight (RFW) and flower diameter

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<sup>-1</sup> day<sup>-1</sup>) was computed using the formula (St<sub>-1</sub>-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). Flower diameter was measured as an index for petals expanding rate. The outer diameter of opened flowers was measured by a Vernier Caliper (mm).

# *Leaf Chlorophyll Index, petal relative water content and total soluble solids (TSS)*

Chlorophyll Index was measured by a SPAD-502 (Minolta Co., Japan). All readings were carried out between the tip and thebase of fully expanded leaves in each sample. 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. For measurement of total soluble sugar content of flower petals sap extract was obtained by squashing the petals. The TSS in the sap was measured with a hand refractometer (Atago model PR 32, Osaka, Japan) during vase life.

# *Electrolyte leakage and membrane stability index (MSI) of petal cells*

For analysis of electrolyte leakage content of petals, one disc  $(1 \text{ cm}^2)$  was punched from each petal and five discs were immersed for 10 min in 50 ml water. The solution was discarded to remove the ions from the cellfree space and after a further addition of 15 ml H<sub>2</sub>O, the discs were shaken (120 rpm) for 90 min on an orbital shaker. The electrical conductivity of the solution C<sub>1</sub> and C<sub>2</sub> was measured by a conductivity meter (644 Conductometer, Metrohm, Switzerland) and then the petal discs were autoclaved for 30 min at 121°C. The leakage rate was expressed as the percentage of total conductance following tissue destruction. Measurement of membrane stability (MSI) was performed by the following formula: Membrane stability index = [1-(C<sub>1</sub>/C<sub>2</sub>)] ×100.

#### 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 using Duncan test at the 0.05 probability level (P<0.05).

### **RESULTS AND DISCUSSION**

**Vase life.** Data presented in Fig. 1A. showed that salicylic acid at concentration of 2 mM with calcium chloride treatment of 5 mM was the most effective treatment in increasing the vase life of cut rose flower cv. Velvet (22 days) followed by salicylic acid 2mM with calcium 2.5 mM and salicylic acid 1mM with calcium 5 mM (17.5 and 17 days, respectively). Also,

salicylic acid treatment at 2 mM with sucrose 2% extended vase life (20 days) significantly (P<0.01) (Fig. 1 B). Ezhilmathi *et al*, (2007) reported that in Gladiolus, the maximum vase-life was obtained once flowers treated with a solution containing 100ppm of 5-SSA + 4% sucrose. Vase-life was improved by using SA in vase solutions. SA extended vase-life of cut rose flowers by regulating water uptake. Improved water balance may be due to possible germicidal activity of SA as an antimicrobial compound acting by inhibiting vascular blockage and/or positive regulatory role of SA on stomatal closure which regulates the rates of transpiration and increases the water-retaining capacity of leaves and petals (Mori *et al*, 2001).



Fig. 1. Effect of  $Cacl_2$  and Salicylic acid pulsing and sucrose holding on vase life of cut rose flower cv. Velvet. Data presented show means±SE (n=3).

### Solution uptake, stem relative fresh weight (RFW) The rate of solution uptake significantly (P <0.05) varied among Cacl2 pulsing and sucrose holding treatments as well as salicylic acid pulsing and sucrose holding treatments (Fig. 2. A and B). The highest amount of solution uptake (3.8 g.stem.day) was obtained in Ca 5mM and sucrose 2% compared to other treatments (Fig. 2 A). The results showed that the water uptake increased significantly (P <0.05) by salicylic acid and sucrose 2% as it reached to the highest level (4.5 g.stem.day) in comparison with other treatments (Fig. 2 B). The highest salicylic acid concentration applied, the greater the improvement in water uptake was observed. Similar results were also reported in cut rose flower (Alaey et al, 2011; Zamani et al, 2011) and gerbera (Kazemi et al, 2011). In the current study, salicylic acid treatments caused to more favourable water uptake than control and suppressed water loss than in the control treatment. This effect of salicylic acid may be due to antimicrobial activity (inhibiting vascular blockage) that increases the water uptake

(Mori et al, 2001) and decrease in transpiration rate (Mei-hua et al, 2008), thereby enhancing water balance of cut flowers. The data in Fig. 2. C and D indicates that the relative fresh weight of cut stems increased gradually till it reached to a maximum value (118 %) for salicylic acid 2mM pulsing and sucrose 2% holding treatment and 115 % for calcium chloride 5 mM pulsing and sucrose 2% holding treatment, respectively during vase period compared to other treatment combinations. The similar results was achieved by Cacl<sub>2</sub>+sucrose + 8-HQS treatment in cut rose flower (Cortes et al. 2011). Kazemi & Ameri (2012) showed that the treated cut gerbera flowers with salicylic acid had the highest levels of relative fresh weight during vase period. Salicylic acid in the vase solution improves cut rose vase life by both delaying senescence and improving water balance (Alaey et al, 2011). This effect can be attributed to the acidifying and stress alleviating properties of salicylic acid, thereby enhancing water uptake and relative fresh weight of cut flowers (Vahdati et al, 2012).

Hassani and Karimi



**Fig. 2.** Effect of calcium chloride and salicylic acid pulsing with sucrose holding on water balance of cut rose stem cv. Velvet. Data presented show means±SE (n=3).

There was significant differences (P <0.01) in flower diameter of cut rose cv. Velvet between sucrose holding treatment in relation to salicylic acid and calcium pulsing treatments (Fig. 3. A and B). The most rate of flower opening was observed in sucrose 2% with SA 2mM (65mm) and Ca 5mM (68mm). Several findings showed that flowers' petal growth is associated with flower bud opening which results from cell expansion (Kenis *et al*, 1985) that requires the influx of water and osmolytes such as glucose into petal cells (Evans and Reid, 1988; van Doorn 1997). According to Chamani *et al*, (2005) shortened vase life was associated with abnormal opening and inhibition of flower opening. Van Doorn (1997) also reported that the decrease in water potential was correlated with inhibition of corolla growth and flower opening.



**Fig. 3.** Effect of calcium chloride and salicylic acid pulsing with sucrose holding on flower opening of cut rose flower cv. Velvet. Data presented show means±SE (n=3).

*Leaf Chlorophyll Index, petal relative water content, total soluble solids (TSS)* 

Leaf chlorophyll index of cut stems was significantly (P<0.05) influenced by SA and Ca pulsing treatments and sucrose holding during vase life. Our results showed that leaf chlorophyll index values of cut rose cv. Velvet increased significantly by SA pulsing alone with maximum value (58.3) in concentration of 2mM and Ca pulsing alone with maximum value of (58.2) in concentration of 5mM. Sucrose holding treatment of 2% with SA pulsing treatment (2mM) and Ca pulsing (5mM) significantly had higher levels of leaf chlorophyll index compared to control (Fig. 4. A and B). In this study, salicylic acid pulsing cut stems had

higher chlorophyll content in comparison to contol. These results were in accordance with findings of Gunes *et al*, (2007) that salicylic acid treatment caused increased chlorophyll content of leaves of barley and maize. Shi *et al*, (2006) reported that foliar application of SA at 1 mM concentration increased chlorophyll content of the leaves of cucumber seedlings grown under heat stress. Leaf senescence can be delayed by exogenous application of calcium due to improved maintenance of chlorophyll and proteins content (Feng *et al.*, 2010). Increase in chlorophyll content after Cacl2 and SA foliar application have been reported (Yildirim *et al*, 2008; Tan *et al*, 2011).



**Fig. 4.** Effect of calcium chloride and salicylic acid pulsing, followed by sucrose holding on leaf chlorophyll index of cut rose flower cv. Velvet. Data presented show means±SE (n=3).

Relative water content of the outer rose petals, in all pulsing treatments of salicylic acid and calcium chloride significantly (P<0.01) varied during vase life. The highest value of petal relative water content in Ca treated flowers was observed in day 6 for Ca 2.5 and 5 mM without sucrose while in SA treatments of 1 and 2 mM with sucrose 2% was in day 9 (Fig. 5. A and B). Sucrose at lower concentrations prolonged the vase life of gladiolus florets by increasing their water uptake but at higher concentrations seemed to impede the uptake, no significant differences were observed among pulsing treatments that comprised long life or the control (Bravdo et al, 1974). They also noted that sucrose decrease water loss from the gladiolus leaves or rose petals, thereby maintaining positive water balance in the flower (Bravdo et al, 1974). Pun & Ichimura (2003) also suggested that the increase in the water uptake by sucrose treatments could be due to the increase in the osmotic concentration of the florets and leaves. Significant differences were observed in terms of petal TSS values among the different pulsing treatments throughout the sampling periods (Fig. 5. C and D). Pulsing treatments of calcium and salicylic acid alone and vase solution was significantly (P<0.01) different on the sixth and nineth day when compared with the control in vase solution without sucrose. Whereas, in vase solution with sucrose 2% there was a significant difference (P<0.05) in TSS values as the control amounts was more than 2.5 and 5mM calcium treatments (Fig. 5. C). In general, there was no consistency of TSS values due to the pulsing treatments during the three sampling periods. One of the important factors that affect longevity of cut flowers during vase life is diminishing of respiratory substrates (Rogers, 1973), whose speed of change depend, at least in part, on the amount of reserves that are present in the flower when they are cut and on the exogenous sugar application to the vase solution (Pun & Ichimura, 2003). Carbohydrates are important reserve compounds, being sucrose the most abundant soluble carbohydrate, sometimes the only one in the phloem sap.



**Fig. 5.** Effect of calcium chloride and salicylic acid pulsing followed by sucrose holding on petal relative water content and total soluble sugar of cut rose flower cv. Velvet. Data presented show means±SE (n=3).

*Electrolyte leakage and membrane stability index of petals* 

There was gradual increase in MSI in both of calcium chloride and salicylic acid pulsing treatments. The

increasing effect of calcium pulsing with sucrose 2% was more significant ((P<0.01) than the same effect of salicylic pulsing in cut rose Velvet cultivar during the vase period (Fig. 6 A and B).



**Fig. 6.** Effect of calcium chloride and salicylic acid pulsing followed by sucrose holding on petal cells membrane stability index of cut rose flower cv. Velvet. Data presented show means±SE (n=3).

Maximum value of membrane stability index (MSI) in cut rose Velvet cultivar was observed in calcium chloride pulsing at 2.5 mM and sucrose 2% holding samples and the minimum value was obtained in control stems both of pulsing treatments without sucrose in holding solution during vase period (Fig. 6. A and B).

Changes in the membrane permeability are strongly related to changes in the composition of the lipid matrix. During the senescence of rose petals, Borochov & Woodson (1989) have shown a relationship between an increase in the sterol phospholipid ratio and a decrease of membrane fluidity. It seems that sterol rigidifies the unsaturated acyl chains of phospholipids inducing a gel phase formation in the membrane and an increase in membrane permeability. Increasing of lipid peroxidation in control over the vase period was supported by the high degree of membrane deterioration expressed as a decrease in MSI.

### CONCLUSION

Pulsing with salicylic acid and CaCl<sub>2</sub> alone and their combinations extended the vase life of cut rose flower cv. Velvet. The most effective concentrations of salicylic acid and CaCl<sub>2</sub> in improving vase life and water balance of cut stem as well as better flower opening during vase period obtained at 2mM and 5mM, respectively. While about their effect on improving the membrane stability index of petal cells of studied cultivar, the concentrations of 1 and 2.5 mM of salicylic acid and CaCl<sub>2</sub> were more beneficial. Their improving effects on the qualitative and quantitative attributes of cut rose stem cv. Velvet increased by the existence of sucrose in the vase solution. According to the results of this experiment, it seems that the application of salicylic acid and CaCl<sub>2</sub> as natural, cheap, safe, and biodegradable compounds can be suitable alternative chemical treatments in order to prolong vase life of cut rose flower.

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