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Full Length Article

The Effects of Calcium on Postharvest Water Status and Vase Life of *Rosa hybrida* cv. Grand Gala

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ABSTRACT

The reduction of the ornamental value of cut flowers is mainly due to their short vase life. The variation in longevity has been associated mainly with vascular obstruction that affects water conduction through the stem. The effect of calcium and 8-Hydroxyquinoline sulfate (HQS) to the vase water was evaluated for different variables including the fresh weight, water flow and longevity of *Rosa hybrida* cv. Grand Gala. The following treatments were tested: $CaCl_2$ (10 mM), Sucrose (Suc) (4%), $CaCl_2$ (10 mM) + Sucrose (4%) and $CaCI_2$ (10 mM) + Sucrose (4%) + HQS (300 ppm). The water consumption, fresh weight, transpiration and the relative water content (RWC) were greater with the $CaCl_2$ + Suc + HQS treatment than that of Sucrose treatment. At the end of the experimental period, the $CaCl_2$ + Suc + HQS treatment induced a greater water flow and the most color infiltration through the vascular tissue compared to the sucrose treatment. The treatment with $CaCl_2$ + Suc + HQS also had the greatest vase life. It is concluded that the combined effect of Ca as a flow resistance reducer and HQS as a germicidal agent contributed to improved vase life. © 2011 Friends Science Publishers

Key Words: Rose; Calcium; RWC; Water flow; Fresh weight; Longevity

INTRODUCTION

The rose is one of the most widely sold and appreciated ornamental flowers in Mexico and throughout the world. However, the life of the rose as a cut flower is short without postharvest treatments due to vascular occlusion resulting from bacterial growth. Although $AgNO_3$ had demonstrated its role like antibacterial agent on vase life of Rosa hybrid (Butt, 2005), however HQS use is of more generalized. Embolism and physiological changes induced in stem affect water movement and subsequent postharvest life (Van Doorn, 1997).

Senescence in the cut flower is affected by three main parameters: the water balance, the supply of carbohydrates and susceptibility to ethylene (Paulin, 1997). Cut flowers those are placed in water frequently develop a water deficit that is caused by xylem occlusion at the basal part of the stem (Dixon & Peterson, 1989).

The use of calcium in vase solutions increases water flow through the stems by association with pectin in the xylem cell walls (Grignon & Sentenac, 1991; Van Ieperen *et al.,* 2006). Calcium interacts with polygalacturonic acid (PGA) groups, forming a structure known as an "egg box", which causes the contraction of pectins in the pit borders, increasing the diameter and consequently, water flow. Van Ieperen *et al.* (2006) found that $CaCl_2$ (1 mM) decreased flow resistance by up to 87% in *Chrysanthemum* stems and by 95% in *Prunus* L., compared to distilled water. On the other hand, calcium increases tissue resistance by slowing senescence because it inhibits the synthesis or action of ethylene (Torre *et al.,* 1999). Capdeville *et al.* (2003) observed that when *Rose* cv. Kiss was kept in conditions in which calcium sulfate was used ($CaSO_4$, 50 mM), increased the longevity by 37% compared to the control.

The life of cut flowers may be enhanced by reducing the loss in fresh weight mainly caused by transpiration losses and maintaining a balance between water flow. The aim of this study was to determine the effect of calcium on the vase life of *Rosa hybrida* cv. Grand Gala, evaluating specifically: water consumption, fresh weight, relative water content, transpiration, water flow and vascular occlusion.

MATERIALS AND METHODS

Cut rose flowers (cv. Grand Gala) were obtained from Villa Guerrero, Mexico. In the laboratory, the roses were rehydrated with distilled water at a pH of 3.5 adjusted with citric acid for three hours. The individual flowers were later selected according to size and weight. In each treatment, five experimental units were used, an experimental unit

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consisted of an intact stem (flower, stem & leaves) that was submerged 16.5 cm into a test tube containing 170 mL of the treatment solution.

Each stem flower was transferred directly to graduated test tube and was kept in along the assay. The following treatments were used: [1] Sucrose (Suc) (4%), [2] $CaCl_2$ (10 mM), [3] $CaCl_2$ (10 mM) + Sucrose (4%) and [4] $CaCl_2$ (10 mM) + Sucrose (4%) + HQS (300 ppm). All of the solutions were adjusted to a pH of 5.5 adjusted with citric acid. Calcium was applied as a hydric stimulant, sucrose as a carbon source and 8-Hydroxyquinoline Sulfate (HQS) as an antibacterial agent.

Water consumption was measured daily in each of the graduated test tubes. The fresh weight of each floral stem was recorded daily. Transpiration (mL cm^{-2}) was calculated as the ratio of the absorbed solution (change in volume) to the total leaf area. The leaf area of each experimental unit was measured at the end of the experiment with a portable LI-COR area meter (model LI-3000A). The corresponding formula was as follows: Transpiration = [(initial volume - final volume)/(leaf area)].

The Relative Water Content (RWC, percent) was determined on the third petal from the outside of the flower, using at least three petals from different flowers for each evaluation. Ten disks measuring 5 mm in diameter were cut from the petals. For each disk a fresh weight, turgid weight (through a four hour immersion period in bidistilled water) and a dry weight (24 h at 80°C) were recorded. The following formula was used to calculate the RWC: RWC = [(fresh weight - dry weight)/(turgid weight - dry weight)] x 100 (Kramer, 1974).

Vascular occlusion was measured using a color infiltration technique (Put & Jansen, 1989) in three of the experimental units (stem, leaves & flower), taking into consideration that the water flow is inversely proportional to the vascular blockage. The floral stems were placed in 1% filtered safranin for one hour under two incandescent light bulbs measuring 200 watts to stimulate transpiration. The height reached by the dye was measured by making transverse cuts in the stalk at intervals of 5 cm. This evaluation was carried out at the beginning and at the end of the experiment, in order to compare flow efficiency.

Stems were prepared for water flow analysis by first removing 5 cm of the base under distilled water. The cut stems were then connected through a latex hose fragment to a hydrostatic column with an approximate pressure of 0.0133 MPa. The solution volume that flowed through the stalk segment corresponding to each treatment was measured every 10 min (Fig. 1). Visual longevity was evaluated daily using photographic records. The condition of the flowers were evaluated on a scale of 1 to 5, representing ornamental to senescent states (Fig. 2). Both the percentage and the duration (in days) of the flowers that were in an ornamental state (state 3, half-open flower) in each treatment were determined.

Statistical analysis: The results with exception of vase life were analyzed with SAS program applying an ANOVA and an associated Tukey's test. For the longevity results, the Kruskal-Wallis test ($P \leq 0.05$) was applied. The last tests were used to determine differences between means.

RESULTS

Water consumption was not affected by any of the treatments on day one. On day two, the calcium treatments of $CaCl_2$ + Suc + HQS and $CaCl_2$ increased water use with values of 13.2 and 12.6 mL, respectively. There were no significant differences between these calcium treatments. The treatment with sucrose had the lowest water consumption (9 mL) and it was significantly lower than the $CaCl_2$ + Suc + HQS and $CaCl_2$ treatments (P = 0.012 & 0.003, respectively). Plants in the treatment with $CaCl_2$ + Suc had a water consumption of 11.6 mL. On days third and fourth, the greatest water consumption obtained was in the treatment with $CaCl_2$ + Suc + HQS, with a value of 13.8 mL. This value was significantly greater than the one obtained in the treatments with $CaCl_2$ + Suc (P = 0.018) and sucrose (P = 0.0024) (Fig. 3). The remaining treatments did not show significant differences in water consumption with respect to sucrose treatment.

The greatest total fresh weights in decreasing order, were obtained with the treatments of $CaCl_2$ + Suc + HQS and $CaCl_2$ + Suc. These weights were statistically superior to the weight recorded with the sucrose-only treatment during the five-days evaluation. Starting on the fourth day, the total fresh weight obtained in each of the other treatments was significantly greater than the sucrose treatment weight (Fig. 4).

The lowest fresh weight recorded for the flower head on the fifth day was obtained from the treatment with sucrose, whereas in the treatment with $CaCl_2$ + Suc + HQS, a significantly higher value was observed. With the exception of the treatment with $CaCl_2$ + Suc, all other treatments were statistically different from the sucrose treatment, which obtained the lowest weight. Regarding the treatment with $CaCl_2$ + Suc + HQS, the weight recorded was significantly higher in relation to the rest of the treatments (P interval between 0.00022 & 0.04313) (Fig. 5).

The transpiration showed a greater flow tendency in the treatments with $CaCl_2$ + Suc + HQS and $CaCl_2$ compared with the sucrose treatments (P = 0.042 & 0.032, respectively). The treatment with $CaCl_2$ + Suc was similar to the rest of the treatments (Fig. 6).

The RWC in the petals on the fifth day was significantly greater in the treatments with $CaCl_2$ + Suc + HQS and $CaCl_2$ compared with the sucrose treatments (P = 0.00183 & 0.00359) and $CaCI_2$ + Suc (P = 0.012 & 0.02485) (Fig. 7).

Fig. 1: The hydrostatic column representation used to measure water flow in the segments of the rose stalks

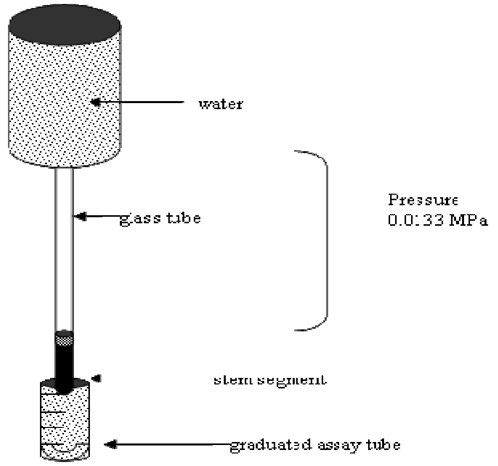
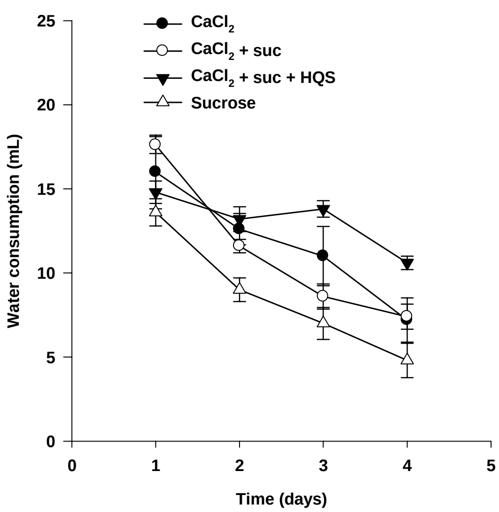


Fig. 2: Flower states of *Rosa* cv. Grand Gala

Fig. 3: The effect of vase solutions on the water consumption of *Rosa* cv. Grand Gala, each point is the mean of 5 stems and bars represent ± SE



Water flow in the stem segments at the beginning of the study did not show any significant differences between treatments (Fig. 8). At the end of the experimental period, differences were found only between the treatment with $CaCl_2$ + Suc + HQS and the sucrose treatment.

Vascular occlusion analysis showed that the color infiltration through the vascular tissue was at the initio comparing with color infiltration on the fifth day showed

Fig. 4: Fresh weight of stem flower evaluated daily, each point is the average value of five replications and the bars represent standard error, the statistical differences obtained for each day are mentioned in the text

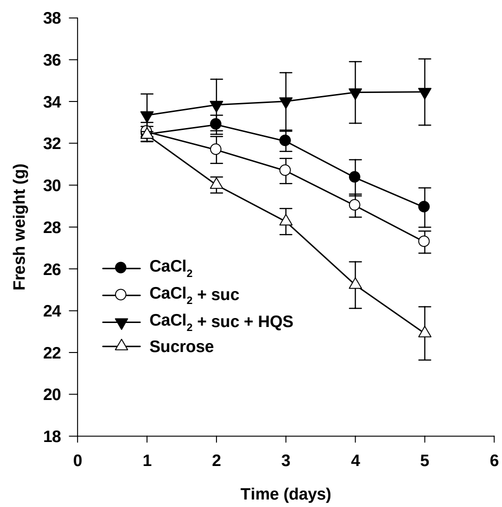
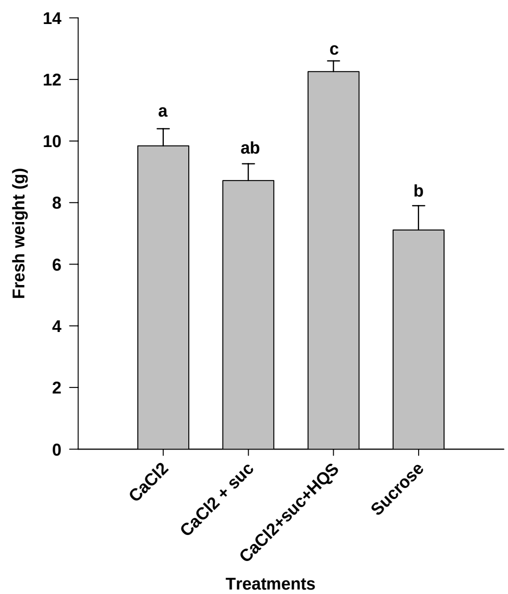


Fig. 5: Fresh weight of the "flower head" measured on the fifth day of vase life, each bar represents the average of five replications with standard error; different letters indicate significant differences according to Tukey's test (*P* < 0.05)



that the treatment with $CaCl_2$ + Suc + HQS as the highest. The calcium treatment had second highest color infiltration and the treatments with sucrose and $CaCl_2$ + Suc were similar (Fig. 10).

The longest vase life was observed in the $CaCl_2$ + Suc + HQS treatment, with a vase life of seven days (Fig. 2). In

Fig. 6: Lysimetric transpiration of *Rosa* cv. Grand Gala, each bar represents the average value of the five replications with its corresponding standard error; different letters indicate significant differences according to Tukey's test ($P \leq 0.05$)

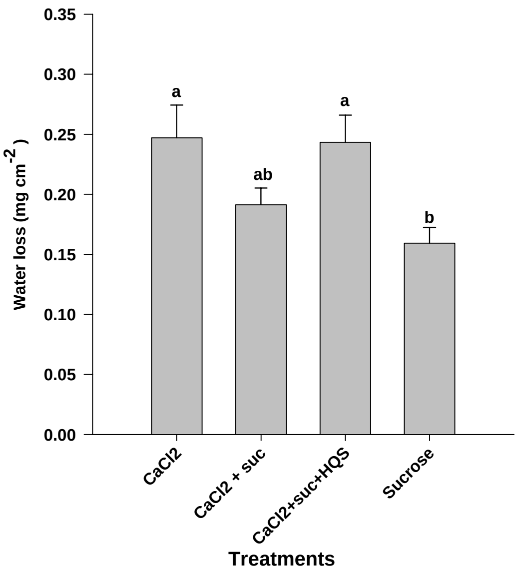
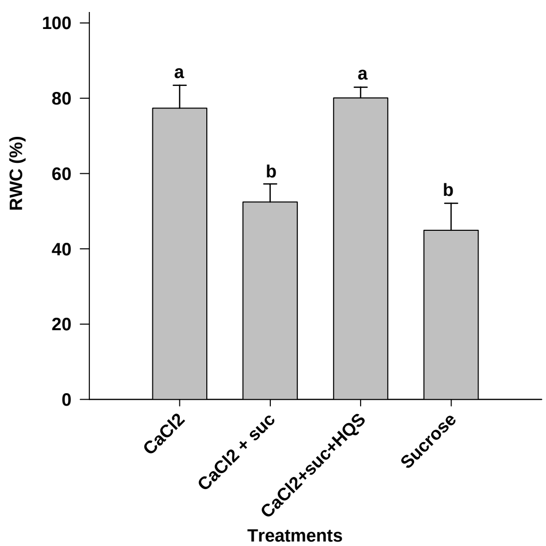


Fig. 7: Relative water content evaluated in the petals during the fifth day, each bar represents the average value of the five replications with its corresponding standard error; different letters indicate significant differences according to Tukey's test ($P < 0.05$)



this treatment 100% of the flowers were in state 3 at the fifth day (Table I), compared to the treatment with $CaCl_2$ in which only 40% of flowers were in state 3. The treatments with sucrose and $CaCl_2$ + Suc did not maintain any flowers in state 3 through the fifth day. Flowers on the fifth day were only in state 4 (80%) and state 5 (20%). In the treatment with sucrose, there appeared to be some bent-neck characteristics in the flower, premature dehydration of the leaves and the petals, non-opened buttons and necrotic edges in petals.

Fig. 8: Cumulative water flow through the 5 cm rose stem segments at the beginning of the experiment, each point is the average value of the five replications and the bars represent standard error

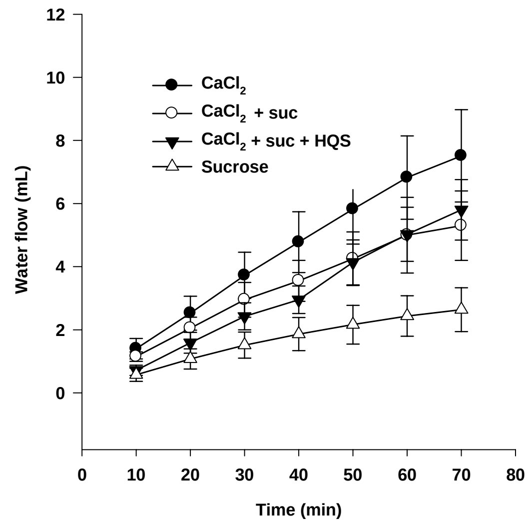
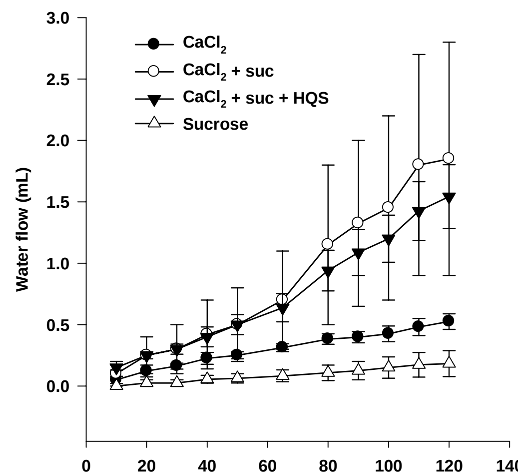


Fig. 9: Cumulative water flow through 5 cm rose stem segments at the end of postharvest life, each point is the average value of the five replications and the bars represent standard error



DISCUSSION

The treatment with HQS improved water consumption and this was likely due to its acidifying effect that inhibits vascular occlusion caused by microorganisms (Ichimura *et al.,* 2005). This result is consistent with the findings reported by Ichimura *et al*. (2006), who recorded a decrease

Fig. 10: Color infiltration in the rose stalks at the beginning of the experiment (first bar) and at the end of the experiment (fifth day), color infiltration allowed for the evaluation of vascular occlusion in the third stage treatments, each bar represents the average value of the five replications with corresponding standard error, different letters indicate significant differences according to Tukey's test (*P* < 0.05)

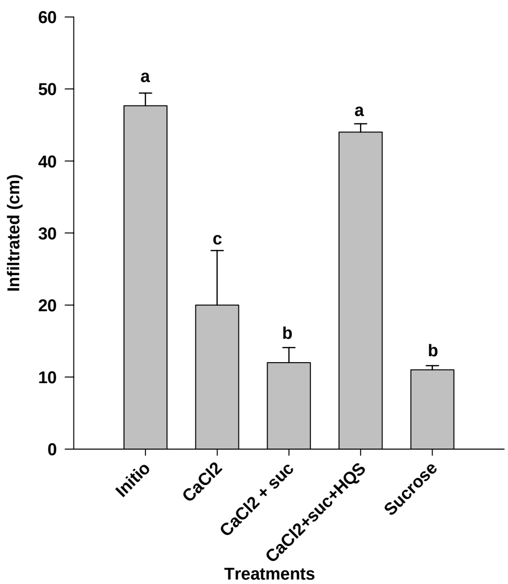


Table I: Percentage of *Rosa* cv. Grand Gala in state 3 on the fifth day, different letters indicate significant differences (*P* < 0.05) through the Kruskal-Wallis test, the remaining treatments did not present any flowers in this state

Treatment	% of flowers in state 3 on day 5	Duration (days)
$CaCl_2$	40 a	5
$CaCl_2$+Suc+HQS	100 b	7

in water consumption by *Rose* cv. Rote Rose treated with sucrose and fructose. In turn, the absence of differences in the treatment with $CaCl_2$ compared to $CaCl_2$ + Suc + HQS is explained by the combination of sucrose with HQS, which acts as an inhibiting agent for bacterial growth.

This study showed that the highest fresh weight at the end of the experimental period was seen with the $CaCl_2$ + Suc + HQS treatment. This result indicates the effectiveness of calcium in maintaining the integrity of cellular membranes and the importance of HQS as an antibacterial agent, allowing a constant hydration and thus inhibiting vascular occlusion. This treatment was related with a better ornamental appearance of the roses. These results are similar to those reported by Ichimura *et al.* (2001), in which 24 of the 25 tested rose cultivars, including Grand Gala, increased to maximum fresh weight and had diminished weight loss with treatments of Sucrose + HQS, when compared to the treatments of sucrose alone. From the previously stated results and findings, it is suggested that a greater longevity can be associated with the maintenance of a high, or at least a constant, fresh weight during the postharvest life of flowers.

The treatment with sucrose alone showed a reduction in the transpiratory rate that, according to Marousky (1969 & 1972) and De Stigter (1980), is explained by stomatal closure. Another explanation for the transpiration decrease, when using sucrose in the solution may be due the reduction in water absorption, since the flow rate is slower, when sugars are in the solution (Durkin, 1979). The presence of an antibacterial agent (HQS) in the treatment with $CaCl_2$ + Suc + HQS, or the absence of a carbohydrate in the treatment with $CaCI_2$, favored the transpiration rate and maintained foliage hydration, as well as the ornamental quality of the roses, for a longer time. The treatment with $CaCl_2$ + Suc + HQS maintained a greater RWC compared to the treatments with sucrose without HQS. This may be due to the effect of this antibacterial agent, which prevents vascular blockage, allowing greater hydration, both in the leaves and in the flowers.

Regarding the treatment with $CaCl_2$, it is assumed that the absence of a carbon source inhibited bacterial growth, thus increasing RWC. Mortazavi *et al.* (2007) reported that the addition of calcium to vase roses increased the relative water content in petals, the water flow in the stalk segments tended to be greater with the $CaCl_2$ (10 mM) treatment at the beginning of the experiment. This suggests a greater effectiveness of this cation as a water stimulant, which confirms the results reported by several authors, where calcium ions have been shown to change the water conductance of stem segments (Van Ieperen *et al.,* 2000; 2006). This change is attributed to the negative charges present in the xylem cell walls that interact with cations such as Ca^{2+}, K^+, Na^+ and Mg^{2+} because of the dissociation of polygalacturonic acids that form COO^- groups. This increase in the effect of conductivity is more evident with the use of divalent cations (Grignon & Sentenac, 1991), together with a greater contraction effect of the walls of the xylem and a reduction in water flow resistance. In this study, the rate of water flow at the end of the experiment was greater in the treatment with $CaCI_2$ + Suc + HQS than in any other treatments. This indicates that the effectiveness of the flow is not the same in recently cut stems, when compared to stems those remained in solutions during 5 days, as the role of HQS is decisive in preventing vascular occlusions caused by bacteria. Ichimura *et al.* (1999) reported similar results, showing that HQS maintains water conductance during seven days in rose stems. Ichimura *et al*. (2006) also reported that water conductance did not decrease in rose stems treated with glucose and HQS. In present study, the greater vascular blockage was observed in the sucrose treatment and this finding are in line with those of Reddy (1988), who concluded that vascular system blockage in cut roses is characterized by a bent-neck in the flower and subsequent dehydration that produces a rapid senescence.

In our results the longevity of Roses (cv. Grand Gala) treated with sucrose and HQS was 7.0 days, which is similar to the findings of Butt (2005), who concluded that inadequate doses of sucrose (excessive or lower) influence the vase life adversely. This author found that with 25 g L^{-1} obtained a vase life of 7.8 days in two cultivars of *Rosa hybrid* (Trika & Whisky Mac). This was consistent with the literature, where Ichimura *et al.* (2001) reported approximately 6.7 days for this same cultivar. It has also been reported that the addition of calcium chloride to the solution increases the vase life, since calcium promotes flower opening, increases fresh weight and delays weight loss. In addition, it delays protein and phospholipid destruction in the petal membranes and eliminates ethylene production (Torre *et al.,* 1999).

In general, the greatest longevity was associated with higher values in the water variables related to water consumption, transpiration, RWC and water flow. Longevity was also associated with the lower values in vascular blockage, a condition associated with the treatment with $CaCl_2$ + Suc + HQS.

CONCLUSION

In this study, the most effective treatment to delay senescence in Rosa hybrida cv Grand Gala is $CaCl_2$ + Suc + HQS used with a pH of 5.5. This treatment provided a 7 days vase life, higher values in water consumption until the last day of evaluation, a delay in fresh weight loss and turgence in the petals and the leaves, as well as greater percentage of opened flowers. Based on the results obtained, it has been established that the critical factors for the conservation of postharvest life in *Rosa* cv. Grand Gala were the calcium ion and the presence of HQS. The use of these chemical would enhance the vase life of roses.

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