Colegio de Postgraduados

Pre and postharvest treatments to reduce the chilling injury in *Lilium* stems

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ABSTRACT

Objective: To increase cold tolerance in floral stems of *Lilium* sp. 'Indian Summerset' stored for two and four weeks using pre- and postharvest treatments.

Design/Methodology/Approach: In the first phase, ascorbic acid (AA; 4 mM), glycine betaine (GB; 100 mM), and distilled water (T) were applied at preharvest and pre-conditioning was carried out at postharvest 0 °C for 24 h, then the steems were stored (6 °C; 85% RH). In the second phase, pulse solutions of AA, GB, or T were applied for 24 h after harvest and stored (7 °C; 92% RH) for two weeks. The variables evaluated were: fresh weight, solution absorption, foliage yellowing, floral opening, flower color, and vase life (VL).

Results: Cold storage causes leaves yellowing, deformation, flower color fading, and reduces VL.

Findings/Conclusions: Preharvest treatments did not have a significant effect on cold tolerance in lilies. In the second phase, the AA pulse solution delayed floral opening and maintained the flowers color, while GB only reduced the leaves yellowing.

Keywords: *Lilium* sp., ascorbic acid, glycine betaine, pre-conditioning, cold damage scales.

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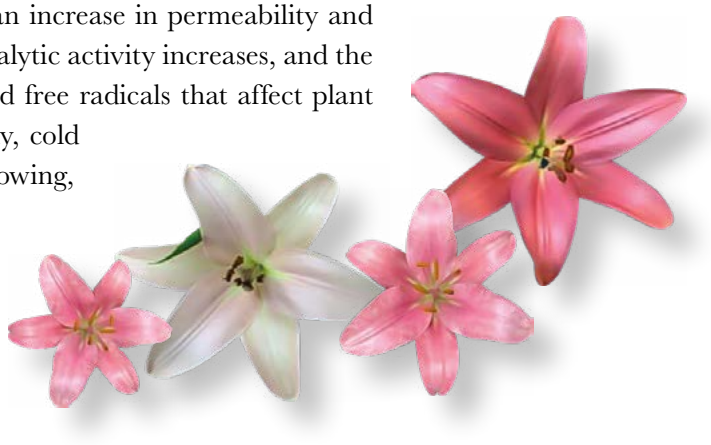
INTRODUCTION

Plants of the genus *Lilium* are primarily divided into *Longiflorum* hybrids, with white, large and aromatic flowers; Asian hybrids, which are great in variety of tepal colors and their resistance to pathogens; and the Oriental hybrids, which have a late floral opening with large and aromatic flowers (Dhiman *et al.*, 2022). To satisfy the demand on specific dates or to transport them, growers store the stems at low temperatures to delay senescence and increase the shelf life of the flowers (Cavalcante *et al.*, 2021).

However, in lilies, it has been reported that storage at temperatures close to 5 °C for more than two weeks causes "chilling injury". This physiological disorder begins with the peroxidation of fatty acids in the cell membrane, so its liquid-crystalline state changes to a solid-gel, causing an increase in permeability and electrolyte leakage. Consequently, catalytic activity increases, and the accumulation of toxic metabolites and free radicals that affect plant tissue begins (Wu *et al.*, 2024). Finally, cold damage in lilies causes leaves yellowing, tepals deformation, and buds abscission (Wei *et al.*, 2018; Darras, 2020).



To increase the tolerance to chilling injury in plant tissues, physical and chemical treatments can be applied. Among the physical ones is the pre-conditioning, which consists of subjecting the tissue to a stress event for short periods of time at temperatures close to 0 °C (Wu *et al.*, 2024). This event produces stress that generates the biosynthesis of hormones and heat shock proteins with the consequent accumulation of antioxidants (Li *et al.*, 2020). This technique is applied in the postharvest handling of fruits such as tomatoes (Zhou *et al.*, 2014) and citrus (Strano *et al.*, 2022). On the other hand, within chemical treatments, there is the application of ascorbic acid (AA) and osmolytes such as glycine betaine (GB) (Wu *et al.*, 2024). AA is an antioxidant able to eliminate radicals such as superoxide and hydroxyl; it decreases lipid peroxidation, protect the selective permeability of the cell membrane system and is a cofactor of ascorbate peroxidase (APX) that reduces H_2O_2 to H_2O (Celi *et al.*, 2023). In tomato plants, pretreatment with AA (0.5 mM) increased the tolerance to freezing stress (4 °C for 7 h), turgor and chlorophyll content in leaves; decreased electrolyte leakage (from 10.5 to 10%), and H_2O_2 content (from 90 to 70 nmol g^{-1} fresh weight) (Elkelish *et al.*, 2020). In anthurium 'Fire Glow', the postharvest application of AA (2-4 mM) decreased chilling injury on the spathe, presenting less darkening and electrolyte leakage (from 63 to 47%) and the activity of enzymes phospholipase and lipoxygenase compared to the control. It also increased the activity of antioxidant enzymes such as APX and catalase (CAT) (Mohammadi *et al.*, 2023).

Glycine betaine (GB) helps to stabilize proteins and activate antioxidant enzymes in stress situations (Fedotova, 2019). In banana (*Musa acuminata* 'Giant-Dwarf'), the preharvest application of GB (100 mM) reduced chilling injury caused by storage at 10 °C (6 h) by delaying the darkening of the epidermis for 8 d, it was observed lower polyphenol oxidase activity and lower electrolyte leakage; it also decreased the ethylene production and delayed the proteins and chlorophyll degradation (Rodríguez-Zapata *et al.*, 2015). In peach fruits, immersion in GB (100 Mm, 10 min) before cold storage (0 °C, 35 d) increased phenylalanine ammonia lyase activity and flavonoid content, reducing chilling injury and delaying senescence (Wang *et al.*, 2019). However, studies regarding the increasing of the cold tolerance in cut flowers are limited, therefore the aim of this work was to know the efficiency of the pre and postharvest application of AA and GB and the effect of pre-conditioning in floral stems of *Lilium* sp. 'Indian Summerset' stored at low temperatures.

MATERIALS AND METHODS

Lilium 'Indian Summerset' stems cultivated in a commercial orchard were used. The experiment was divided into two experiments. The first experiment preharvest applications of AA (4 mM), GB (100 mM), and distilled water (control 2, T2) were performed. Four preharvest sprays (10 mL per stem) were applied to the floral buds at 90, 95, 100, and 105 days after bulb sowing and the next day (day 106) the stems were harvested. Then 30 floral stems were selected, ten for each treatment (each stem was considered a replicate). On the other hand, another 10 stems were taken to carry out the pre-conditioning treatment (0 °C, 24 h). Finally, the stems were transported to the cold room to store in water for two and four weeks (6 °C and 85% RH).

In the second experiment, stems of lilies with the first bud flower with turning color were harvest and transported to the laboratory, then were trimmed and place in a vase with one of the following solutions: AA (4 mM), GB (100 mM), and distilled water (T2) for 24 h, and subsequently the solutions were replaced with tap water, and the stored at 7 °C and 92% RH during two weeks. In this phase, other floral stems were left at room temperature (control 1, T1) to corroborate their potential for vase life. For each treatment, 16 stems were used (each stem was considered a replicate).

After cold storage, the stems were placed in glass bottles with 250 mL of tap water and trimmed to 60 cm length, removing 20 cm of leaves from the stem base. The bottles were placed in a room at 21 °C and 76% RH with 12 h light and 12 h darkness. The variables evaluated were the following:

- Visual appearance of the flower stems. Photographs of flower stems were taken during vase life (VL) with a 12 MP iPhone® XR wide-angle camera to identify the symptoms of chilling injury.
- Changes in fresh weight (%). The stems were weighed before and after the cold storage period. When placed in vases, they were weighed daily with a digital scale (Esnova® SE-2000, with 0.1 g precision), and weight changes were calculated based on the difference between the initial and final weight.
- Water uptake (mL/ tallo). The solution consumption by the flower stems was calculated based on the difference in weight of the solution daily.
- Leaves color. A scale from green (1) to yellow (6) was established and measured daily in the leaves during VL.
- Flowers color. Using a precision colorimeter NR20XE (Shenzhen ThreeNH Technology, China), the L*, a*, and b* values of the flowers were obtained to calculate the Hue° angle and the chroma value.
- Floral opening (%). The total number of flower buds was counted when the stems were harvested and after storage the opening percentage was calculated.
- Vase life. After storage, the days in which the flower stems remained without showing symptoms of senescence such as wilting, petals abscision, or leaves yellowing were counted. The end of vase life was considered when 50% of the flowers were wilted and most of the leaves showed chlorosis.

Data analysis

The experimental design was completely randomized. In the first phase were used 5 replicates per treatment per cold storage period, and in the second one, 16 replicates. The data were analyzed with ANOVA and significant differences were determined with the Tukey test (α=0.05); where appropriate, nonparametric tests were used.

RESULTS AND DISCUSSION

First experiment: Preharvest treatments and pre-conditioning

No significant differences were found in the variables evaluated after two or four weeks of storage (6 °C and 85% RH) with preharvest treatments (AA, GB) and pre-conditioning

(0 °C for 24 h) and the control in *Lilium* 'Indian Summerset' to increase the tolerance to chilling injury. These results contradict what was reported in gerbera, where pre (1 mM) and postharvest (5 mM) applications of spermine and aminobutyric acid showed that the preharvest treatment was more effective in prolong the VL (Mohammadi *et al.*, 2020). In peonies, preharvest silicon applications were more effective in improving stem quality than the postharvest applications (Song *et al.*, 2021).

It is possible that the lack of effectiveness of these compounds was because foliar spraying requires higher concentrations of the compound. For example, Mohammadi *et al.* (2023) applied AA (0-4 mM) to anthurium stems 'Fire Glow' by foliar spray and pulse solution before storing at 4 °C. With foliar spraying, the greatest increase in VL (25 d) was achieved with 4 mM, while with the pulse solution, it was achieved with 2 mM (27 d), control stems last only 18 d.

Also it is possible that lilies come from a bulb that functions as organ of reserve, being the main source of nutrients during the stem growth and development (De Hertogh, 1992). Due to the relevance of the bulbs in *Lilium* plants, it would be important to verify that preharvest treatments would be applied directly to the bulb and not to the plant, just as has been reported for some growth retardants such as paclobutrazol that have resulted in high effectiveness (Torres-Pio *et al.*, 2021; Rios-Florida *et al.*, 2022).

In *Lilium* 'Indian Summerset', storage at 6 °C and 85% HR for two weeks caused leaves yellowing, flower buds deformation and abscission also to color degradation in the flowers as a symptom of chilling injury. Regardless of the treatment and the storage period at 6 °C, at least six color tones were observed in the flowers and four levels of deformation (Figure 1).

Second experiment: Application of pulse solutions

Pulse solutions with AA or GB decreased the loss of flower color caused by cold storage. After cold storage no differences in the color tone of the flowers (Hue° value), were recorded

Figure 1. Color degradation (A) and deformation (B) of the tepals in flowers of *Lilium* 'Indian Summerset' caused by storage at 6 °C and 85% RH. The scale goes from 1 (mild damage) to 4-5 (severe damage). T1: unstored stems.

but the color intensity (Chroma value) was affected. The treatment that had values similar to the control (T1) was AA, while the greatest color loss was observed with GB (Figure 2A). At the beginning of the VL, the flowers did not show petals deformation until day five; flowers with GB treatment showed a heterogeneous pattern in the color and waviness of the tepal margin (Figure 2B and C).

During cold storage ethylene biosynthesis increases and accelerates senescence provoking color fading (Nergi and Arndt, 2023). Ethylene activates transcription factors that induce the expression of genes that encode enzymes that degrade pigments such as peroxidases (Dias *et al*., 2021). On the other hand, it is normal that during floral opening the cell wall of the epidermal cells in the tepals undergoes modifications in its structure and shape. However, a stress situation, such as cold storage, causes water loss and, therefore, loss of turgor, which makes deformation and changes in the shape of the cells (Zhang *et al*., 2021).

Fresh weight (%): During cold storage, the stems of *Lilium* 'Indian Summerset' increased their fresh weight above 100%, compared to the initial value. During the VL, no significant differences were found in the stored stems, but they had greater weight gain than the unstored stems (T1). Furthermore, fresh weight began to decline on day five with T1, on day three in T2, and on day two in AA and GB treatments, after cold storage. This weight loss is important as it marks the point at which senescence is noticeable (Figure 3).

Woltering and Paillart (2018) stored rose stems for two days at 6 °C and for 28 days at 0.5 °C, after storage the stems were placed in water or in hydroxyquinoline citrate solution (8-HQC, 150 mg L^{-1}). Regardless of the solution, cold storage decreased fresh weight gain compared to unstored stems. Despite this, the use of 8-HQC improved the quality of the stems, since, being a compound with an acidic pH, it prevents the growth of microorganisms at the base of the stem and maintains the water conductivity through the xylem, resulting in greater weight gain compared to water alone.

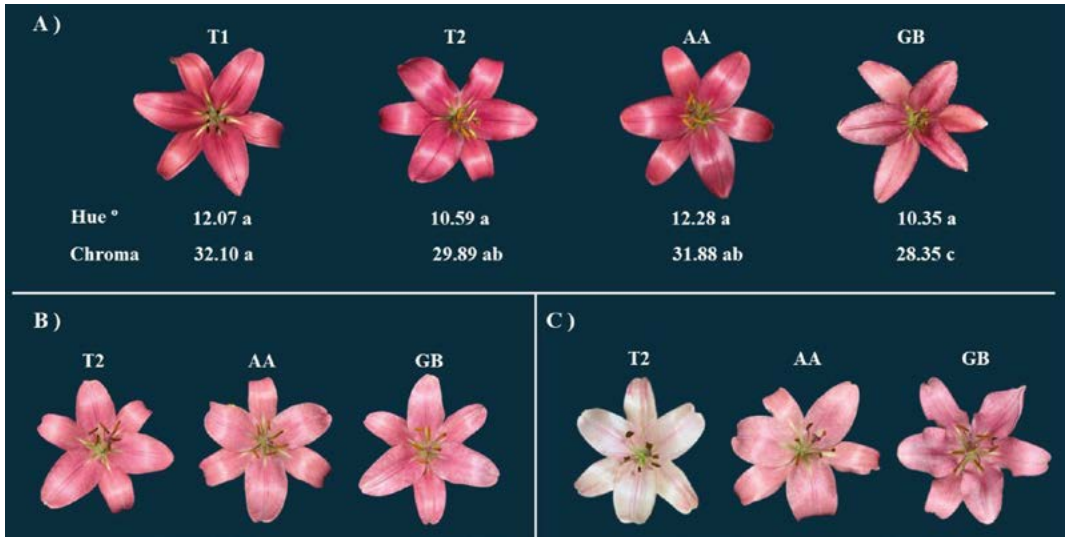


Figure 2. Color of *Lilium* 'Indian Summerset' flowers after storage at 7 °C and 92% RH for two weeks (A) and appearance of the best (B) and worst flowers (C) of each treatment on day five of the vase life. T1: unstored stems, T2: cold storage, AA: ascorbic acid (4 mM); GB: glycine betaine (100 mM).

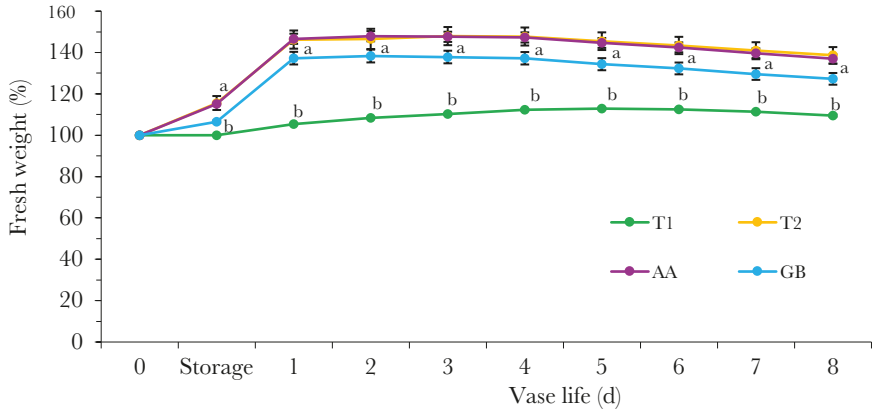


Figura 3. Changes in fresh weight of *Lilium* 'Indian Summerset' stems after two weeks of storage at 7 °C and during vase life. T1: unstored stems, T2: control (cold storage), AA: ascorbic acid (4 mM), GB: glycine betaine (100 mM).

Water uptake: *Lilium* 'Indian Summerset' stems stored for two weeks reduced their ability to absorb water. This is because cold storage affects the functionality of the stomata in the leaves. In rose, storage for 28 d at 0.5 °C affected the conductance of the stomata because when the stems were placed at room temperature, transpiration was lower than the unstored stems (Woltering and Paillart, 2018). Something similar occurred in Heliconia where cold storage (13 °C and 84% RH) decreased water consumption from 0.066 to 0.034 mL g^{-1} compared to the stems stored at 20 °C (Carrera-Alvarado *et al.*, 2021).

In *Lilium* "Indian Summerset" it was observed that, during the first three days of VL, no significant differences were found in water consumption. Starting on day four, the stems without cold storage had the highest water absorption, followed by the stems with AA, T2, and GB treatments (Table 1).

For an adequate water flow through the xylem in the floral stems, it is common to use biocidal and low pH compounds to control microbial growth and maintain water conductivity (Arriaga *et al.*, 2020). Ascorbic acid lowers pH and inhibits the oxidation of phenolic compounds and ROS generation, thereby reduces vascular obstruction of stems and increases water absorption (Chen *et al.*, 2024). This explains why the stems with AA treatment (4 mM) absorbed more water during their VL (Table 1).

Leaves color: In general, the leaves of *Lilium* 'Indian Summerset' presented six levels of yellowing during VL. Both treatments (AA and GB) for 24 h decreased and delayed leaves yellowing compared to T2 (Table 1 and Figure 4).

It has been reported that monodihydroascorbate, derived from the oxidation of AA, is an electron carrier in photosystem II. Furthermore, AA maintains the content of photosynthetic pigments, membrane stability, and water potential, regulates ROS, and promotes the antioxidant defense system (Celi *et al.*, 2023). For its part, GB stabilizes the structure and activity of proteins that participate in photosynthesis (Fedotova, 2019).

For this reason, both AA and GB are compounds that maintain the integrity of the pigments. For example, in tomato seedlings, treatment with AA (0.5 mM) increased their cold tolerance (4 °C for 7 h) and maintained chlorophyll concentration (Elkelish *et al.*,

Table 1. Vase life, floral opening, cumulative vase solution consumption, and leaves yellowing scale of *Lilium* 'Indian Summerset' stems stored at 7 °C for two weeks.

	Vase life (d)	Floral opening (%)		Uptake water (mL stem^{-1})		Yellowing scale	
		A	B	4 d	8 d	4 d	8 d
T1	12.13 a*	---	42.57 b	75.56 a	127.26 a	1.00 c	2.00 ab
T2	8.56 b	38.55 ab	50.47 ab	63.02 b	97.80 bc	1.83 a	2.29 a
AA	8.75 b	31.20 b	52.98 a	62.50 b	99.50 b	1.13 b	1.46 b
GB	8.37 b	43.44 a	55.98 a	58.19 b	85.69 c	1.2 b	1.45 b

[z] Different letters indicate significant differences from the Tukey test with α of 0.05. T1, control without cold storage; T2, control with cold storage; AA, ascorbic acid (4 mM); GB, glycine betaine (100 mM); A, upon exiting the two-week cold storage period; B, to the day of the vase's life when the fresh weight began to decline.

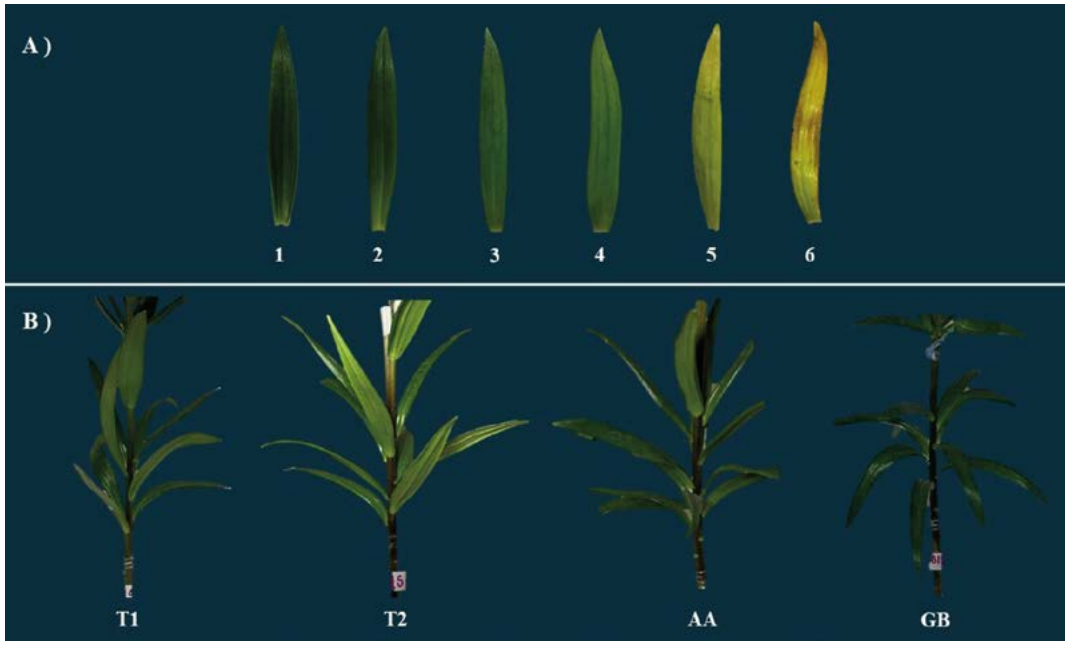


Figure 4. Scale of leaves yellowing on flower stems of *Lilium* 'Indian Summerset' after two weeks of storage at 7 °C (A) and appearance of leaves on day zero of vase life. T1: unstored stems, T2: control (cold storage), AA: ascorbic acid (4 mM), GB: glycine betaine (100 mM).

2020). In *Dalbergia odorifera* seedlings, GB spraying (10-50 mM) maintained the greenery and chlorophyll content (Cisse *et al.*, 2021).

Floral opening and vase life: Refrigerated storage aims to delay the floral opening of the buds and prolong their vase life (Cavalcante *et al.*, 2021). In *Lilium* 'Indian Summerset', storage at 7 °C for two weeks delayed flower opening. While the T1 stems reached their maximum floral opening on day seven and last 14 days, the stored stems reached their maximum floral opening on the second day after storage and had six additional days of VL (total 20 days). The GB pulse solution accelerated floral opening during cold storage (43%) while with AA it decreased (31%) compared to the control (T2) which had 38%

opening upon leaving the storage period. Finally, no significant differences were found in the duration of VL between treatments (Table 1).

Arabia *et al*. (2024) report that AA is an important antioxidant that controls the cellular redox state since it reduces free radicals and is a cofactor of enzymes such as APX. It interacts with ethylene, influencing processes such as senescence. Therefore, the exogenous application of AA can help to tolerate chilling injury disorders and increase the storage life of the product. In lilies, our results showed that AA delayed floral opening and yellowing of the leaves.

CONCLUSIONS

Cold storage of floral stems of *Lilium* cv. 'Indian Summerset' for two and four weeks accelerates the leaves yellowing, and provoked color fading and deformation of the flowers, and reduces the vase life. Preharvest treatments with AA and GB and pre-conditioning at 0 °C did not increased the tolerance to chilling injury in the stems. The postharvest application of AA through pulse solution for 24 h decreased chilling injury, maintain the tepals color, reduce the leaves yellowing and prevent the flower deformation. Studies around AA mode of action are necessary to increase the cold storage length in cut flowers.

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