Ornamental Horticulture
ISSN 2447-536X | WWW.ORNAMENTALHORTICULTURE.COM.BR
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

Article

Effect of calcium chloride and salicylic acid on some morphological, biochemical and postharvest properties of alstroemeria cut flowers 'Orange Queen'

Efeito do cloreto de cálcio e do ácido salicílico em algumas propriedades morfológicas, bioquímicas e pós-colheita de flores cortadas de Alstroemeria 'Orange Queen'

Shahin Teymouri Azar[1] **and Zohreh Jabbarzadeh**[1]*

[1]Urmia University, Faculty of Agriculture, Department of Horticultural Science, Urmia, Iran.

Abstract: This study investigated the impact of varying calcium chloride ($CaCl_2$) and salicylic acid (SA) concentrations on the growth, biochemical, and postharvest quality of Alstroemeria 'Orange Queen' flowers. A completely randomized factorial design (CRD) with two factors and three replications was employed. The first factor involved foliar application of 0, 75, 150, or 225 mg L^{-1} $CaCl_2$, while the second factor consisted of 0, 150, 300, or 450 mg L^{-1} SA. Morphological characteristics (number of leaves, leaf fresh and dry weight, number of florets) and biochemical parameters (photosynthetic pigment content, soluble sugar content) were evaluated. Additionally, postharvest traits (relative fresh weight, relative solution uptake, malondialdehyde content, catalase and ascorbate peroxidase activity) were assessed. The highest fresh and dry weights and the greatest number of florets were observed in Alstroemeria treated with 225 mg L^{-1} $CaCl_2$ and 450 mg L^{-1} SA. This combination also improved the flowers' postharvest quality. Photosynthetic pigment content was significantly enhanced by both $CaCl_2$ and SA, while malondialdehyde content was reduced compared to the control. Antioxidant enzyme activity was significantly increased following the application of $CaCl_2$ and SA. Foliar application of 225 mg L^{-1} $CaCl_2$ and 450 mg L^{-1} SA promoted the growth, biochemical composition, and postharvest quality of Alstroemeria 'Orange Queen' flowers. This treatment improved flower size, number, and enhanced their resistance to stress, suggesting its potential for enhancing the commercial value of this cultivar.
Keywords: malondialdehyde, number of florets, photosynthetic pigments, postharvest quality.

Resumo: Este estudo investigou o impacto de diferentes concentrações de cloreto de cálcio ($CaCl_2$) e ácido salicílico (SA) no crescimento, bioquímica e qualidade pós-colheita de flores de Alstroemeria 'Orange Queen'. Foi empregado um delineamento fatorial inteiramente casualizado (DIC) com dois fatores e três repetições. O primeiro fator envolveu aplicação foliar de 0, 75, 150 ou 225 mg L^{-1} $CaCl_2$, enquanto o segundo fator consistiu em 0, 150, 300 ou 450 mg L^{-1} SA. Foram avaliadas características morfológicas (número de folhas, massa fresca e seca de folhas, número de floretes) e parâmetros bioquímicos (teor de pigmentos fotossintéticos, teor de açúcares solúveis). Além disso, foram avaliadas características pós-colheita (peso fresco relativo, absorção relativa de solução, teor de malondialdeído, atividade de catalase e ascorbato peroxidase). Os maiores pesos fresco e seco e o maior número de flores foram observados em Alstroemeria tratada com 225 mg L^{-1} $CaCl_2$ e 450 mg L^{-1} SA. Essa combinação também melhorou a qualidade pós-colheita das flores. O conteúdo de pigmento fotossintético foi significativamente aumentado tanto pelo $CaCl_2$ quanto pelo SA, enquanto o conteúdo de malondialdeído foi reduzido em comparação com o controle. A atividade da enzima antioxidante aumentou significativamente após a aplicação de $CaCl_2$ e SA. A aplicação foliar de 225 mg L^{-1} $CaCl_2$ e 450 mg L^{-1} SA promoveu o crescimento, a composição bioquímica e a qualidade pós-colheita das flores de Alstroemeria 'Orange Queen'. Este tratamento melhorou o tamanho e o número das flores e aumentou sua resistência ao estresse, sugerindo seu potencial para aumentar o valor comercial desta cultivar.
Palavras-chave: Número de flores, malondialdeído, pigmentos fotossintéticos, qualidade pós-colheita.

Introduction

Alstroemeria, or the Peruvian Lily, is a monocotyledonous plant belonging to the Alstroemeriaceae family. This perennial, native to South America, thrives in tropical regions where its sensitivity to cold allows it to flourish as an herbaceous plant. Commonly grown in pots and gardens, Alstroemeria offers vibrant beauty to both indoor and outdoor spaces (Naseri and Ebrahimi-Geravi, 2002).

More than just aspirin for plants, salicylic acid, with its simple formula ($C_7H_6O_3$), is a versatile phenolic compound with surprising benefits for plant growth. This naturally occurring molecule acts as a powerful plant growth regulator, influencing various aspects of their development. One key function of salicylic acid is its ability to interfere with ethylene biosynthesis, a process that hastens senescence and shortens the life of cut flowers. Studies by Korver et al. (2018) and Perez-Llorca et al. (2019) demonstrate its effectiveness in preventing this process, thereby extending the vase life of cut flowers. Beyond extending life, salicylic acid also acts as a growth stimulant, encouraging flowering, increasing flower longevity, and regulating crucial functions like stomata closure, chlorophyll content, and plant transpiration and respiration (Luo et al., 2022). One key mechanism through which salicylic acid maintains flower health is by preventing chlorophyll degradation. By inhibiting the function of ACC oxidase, an enzyme involved in ethylene production, and regulating abscisic acid, another plant hormone, salicylic acid ensures chlorophyll retention (Ali et al., 2018). As a result, the vibrant colors and overall quality of cut flowers are preserved. Overall, salicylic acid emerges as a promising tool for enhancing plant growth and development. Its diverse functions, from interfering with ethylene biosynthesis to stimulating flowering and maintaining chlorophyll, highlight its potential as a valuable asset in agriculture and horticulture.

Calcium, a key player in plant health, is a large cation (Ca^{2+}) and one of the earth's crust's most abundant elements. Over 60% of a plant's calcium resides in its cell walls, contributing to their strength. However, calcium's slow movement within the plant presents challenges for horticultural crops. This unique distribution involves numerous locations within cell walls and limited movement from the cytoplasm. In the middle lamella, calcium loosely binds to carboxyl groups in pectins (Kholdebarin and Eslamzadeh, 2001). Calcium plays a crucial role in cut flower longevity, delaying senescence and improving preservation by influencing ethylene production. It inhibits ethylene synthesis through its contribution to cell membrane stability, essentially mediating the

https://doi.org/10.1590/2447-536X.v30.e242697 | * Correspondent author: z.jabbarzadeh@urmia.ac.ir | Editor: Claudia Fabrino Machado Mattiuz (Escola Superior de Agronomia "Luiz de Queiroz", Brasil) | Received Oct 27, 2023 | Accepted Mar 18, 2024 | Available online May 6, 2024 | Licensed by CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/)

conversion of 1-Aminocyclopropane-1-Carboxylic Acid (ACC) into ethylene via non-specific membrane enzymes. Additionally, it protects against cell-destroying enzymes like polygalacturonase, further extending the flower's vase life. Beyond these benefits, calcium affects membrane enzymes, interacts with plant hormones in cell walls, and even contributes to pollen tube growth (Barker and Pilbeam, 2007).

Previous studies have explored the diverse effects of salicylic acid and calcium on various plants. Mady et al. (2023) demonstrated that foliar-applied salicylic acid enhanced growth and biochemical characteristics in eggplant (*Solanum melongena* L.), while Shahmoradi and Naderi (2018) found it improved plant height, weight, and antioxidant activity in *Jasminum nudiflorum* under salt stress. Additionally, Reddy and Sarkar (2016) identified positive impacts of calcium nitrate on both vase life and corm production in *Gladiolus communis*. Further, Nazari (2019) reported that both calcium chloride and nano-chelated calcium positively influenced vegetative and reproductive traits, as well as postharvest life, in *Polianthes tuberosa*.

This study builds upon this valuable body of research by investigating the combined effects of salicylic acid and calcium on Alstroemeria 'Orange Queen'. Our aim is to evaluate how these treatments influence growth, biochemical properties, and postharvest quality, potentially uncovering new avenues for enhancing the value of this popular cut flower.

Materials and methods

a) Plant materials, treatments and growing conditions

This study investigated the combined effects of salicylic acid (SA) and calcium chloride ($CaCl_2$) on growth, biochemical traits, and flowering in hydroponically grown Alstroemeria (*Alstroemeria aurea*) 'Orange Queen' plants (Royal Van Zan Ten – Netherlands) (Fig. 1). Plants were obtained from a commercial greenhouse and cultivated in pots filled with a 1:3 perlite-coco peat mixture. At two months old, well-established plants were subjected to foliar sprays of various SA (0, 150, 300, and 450 mg L^{-1}) and $CaCl_2$ (0, 75, 150, and 225 mg L^{-1}) concentrations every two weeks for four months. A completely randomized design (CRD) with three replications, three pots per replicate, and one plant per pot was employed. The greenhouse maintained a temperature of 16-18 °C during the day and 10-13 °C at night, with 10-12 hour days, light intensity of 400-500 µmol m^{-2} s^{-1} (supplemented with fluorescent lamps), and 60%-70% humidity. Plants received 150-200 mL of a customized nutrient solution (details in Table 1) throughout the experiment.

Table 1. Nutritional program used for Alstroemeria for 100 L nutrient solution.

Magnesium sulfate	Potassium nitrate	Ammonium nitrate	Sodium molybdate	Borax	Monoammonium phosphate	Manganese sulfate	Zinc sulfate	Potassium sulfate	Iron chelate 6%	Calcium Nitrate-Ammonium Nitrate
10 g	32 g	4 g	0.035 g	0.03 g	5 g	0.2 g	0.15 g	8g	5g	10 g

Fig. 1. Alstroemeria 'Orange Queen' cut flowers used in this research.

b) Morphological parameters

b.1) Fresh and dry weight of leaves

Fresh and dry weights of leaves were measured. For fresh weight, leaves were weighed on a METTLER PJ300 digital scale with 0.0001 g accuracy. For dry weight, leaves were placed in paper bags, oven-dried at 72 °C for 24 hours, and then re-weighed on the same scale.

b.2)Number of petals and florets

In each plant, the number of petals and florets was counted.

c) Biochemical measurements

c.1) Chlorophyll *a*, *b* and total chlorophyll content

Photosynthetic pigments (chlorophyll *a*, *b*, and total chlorophyll) were quantified following Lichtenthaler's (1987) method. Briefly, 0.1 g of fresh leaf tissue was homogenized in 5 mL of 80% acetone and centrifuged at 2,500 rpm for 10 min. Absorbance readings at 663 nm, 645 nm, and 470 nm were used to calculate chlorophyll *a*, *b*, and total chlorophyll content, respectively, using established equations (Lichtenthaler, 1987).

Chlorophyll *a* (mg g^{-1}) =11.75 (A663) – 2.350(A645) x (V/(1000 x wt))
Chlorophyll *b* (mg g^{-1}) =18.61 (A645) – 3.96(A663) x (V/(1000 x wt))
Total Chlorophyll (mg g^{-1}) =20.2 (OD645) + 8.02(OD663) x (V/(1000 xwt))
Where V is the sample volume (mL), and W is the sample fresh weight (mg).

c.2) Measuring the soluble sugar content of leaves and petals

Total soluble sugars were determined using the method of Irigoyen et al. (1992). Briefly, 0.5 g of leaf or petal tissue was homogenized in 5 mL of 95% ethanol and centrifuged at 3,500 rpm for 15 min. The supernatant was mixed with 5% ethanol 70% and the absorbance was measured at 625 nm after reacting with anthrone in a hot water bath for 10 min.

d) Postharvest parameters measurement

Freshly harvested Alstroemeria cut flowers were immediately placed in tap water and transported to the laboratory. Stems were then re-cut to 40 cm under distilled water, and four stems were placed in each 500 mL vase containing distilled water. The flowers were maintained under controlled conditions throughout the experiment: 22 °C ± 1 °C temperature, 70% relative humidity, and a 12-hour photoperiod with fluorescent lamps providing 13 µmol m^{-2} s^{-1} light intensity. Samples were collected from the flowers on days 0, 6, and 12.

e) Malondialdehyde

Malondialdehyde (MDA) content was measured following Cakmak and Horst (1991). Briefly, 0.2 g of petal tissue was homogenized in 5 mL

of 1% TCA and centrifuged at 8,000 rpm for 10 min. The supernatant was mixed with a solution of 20% TCA and 0.5% TBA (4:1 ratio) and incubated in a 95 °C water bath for 30 min, followed by immediate cooling in an ice bath. After another centrifugation at 8,000 rpm for 5 min, absorbance readings were taken at 600 and 532 nm. MDA content was calculated in µmol/g fresh weight using the provided formula.

$$\mathrm{MDA}(\mu\mathrm{mol/g\ FW}) = (\mathrm{A532} - \mathrm{A600}/155)^{*}100$$

f) Catalase and Ascorbate Peroxidase enzymes activity

For antioxidant enzyme activity assays (catalase and ascorbate peroxidase), 0.5 g of leaf tissue was homogenized in 5 mL of pH 7.5 extraction buffer (50 mM Tris, 3 mM $MgCl_2$, 1 mM EDTA). For ascorbate peroxidase activity, 0.2 mM ascorbate was added to the buffer. The homogenate was centrifuged at 4 °C and 4,000 rpm for 20 min (Kang and Saltveit, 2002). Catalase activity was measured at 240 nm using Aebi's method (1984), and ascorbate peroxidase activity was determined at 290 nm using Nakano and Asada's method (1981).

g) Statistical analysis of data and software used

This experiment utilized a completely randomized factorial design with three factors: salicylic acid (0, 150, 300, and 450 mg L^{-1}), calcium chloride (0, 75, 150, and 225 mg L^{-1}), and sampling time (days 0, 6, and 12 of vase life). Data analysis was performed using SAS version 9.2, with means compared using Tukey's multiple comparison test at a 1% significance level.

Results and Discussion

a) Number of leaves

Foliar application of calcium chloride and salicylic acid increased Alstroemeria leaf number; however, the increase was only significant for treatments with 300 mg L^{-1} salicylic acid and 225 mg L^{-1} calcium chloride and also a concentration of 450 mg L^{-1} salicylic acid with 150 or 225 mg L^{-1} calcium chloride (Fig. 2).

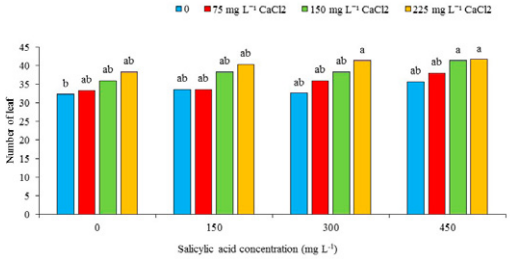

Fig. 2. The effect of various concentrations of calcium chloride and salicylic acid on number of leaves for Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.05$).

b) Leaf fresh weight

Foliar application of calcium chloride and salicylic acid increased leaf fresh weight in some treatments compared to the control. Among tested concentrations, only 450 mg L^{-1} salicylic acid with 75 or 150 mg L^{-1} calcium chloride and 150 mg L^{-1} salicylic acid with 225 mg L^{-1} calcium chloride significantly increased leaf fresh weight (Fig. 3).

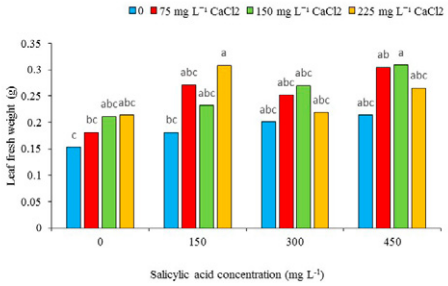

Fig. 3. The effect of various concentrations of calcium chloride and salicylic acid on leaf fresh weight for Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

c) Leaf dry weight

Foliar application of calcium chloride and salicylic acid increased leaf dry weight, but only significantly for plants treated with 450 mg L^{-1} salicylic acid and 75 mg L^{-1} calcium chloride, which showed an 81% increase compared to the control (Fig. 4).

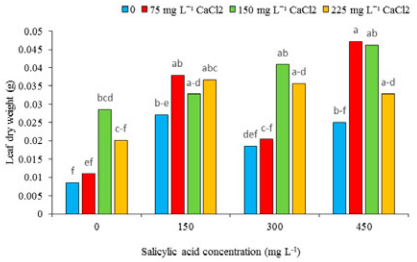

Fig. 4. The effect of various concentrations of calcium chloride and salicylic acid on leaf dry weight for Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

d) Number of Florets

Various concentrations of calcium chloride and salicylic acid increased the number of florets compared to the control, with a maximum of 8.33 florets observed for plants treated with 225 mg $^{-1}$ salicylic acid and 300 or 450 mg L^{-1} calcium chloride (Fig. 5).

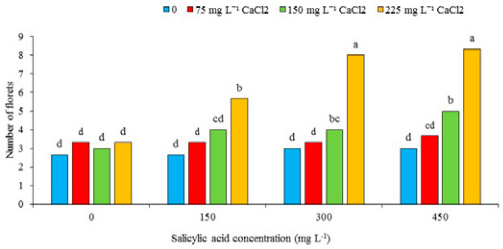

Fig. 5. The effect of various concentrations of calcium chloride and salicylic acid on number of florets for Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

The study found that salicylic acid and calcium chloride improved several growth parameters, such as the number of leaves and their fresh and dry weight. Calcium is a crucial element for maintaining and enhancing the quality of cut flowers. It's a component of plant cell walls, and research has shown it plays a vital role in forming strong polymer bonds within the tissue, specifically between membrane pectocellulosic components. This translates to increased mechanical strength of the cell walls (Tofighi Alikhani et al., 2021). Calcium's primary function is to stabilize cell walls and membranes, while also contributing to cell growth. Additionally, it helps balance cations and anions, activates specific enzymes, and regulates osmotic pressure. Calcium also promotes healthy root growth and function. It protects plants against mechanical damage caused by wind and harsh weather conditions (Moallaye Mazraei et al., 2020). Furthermore, calcium significantly influences signal transduction mechanisms in plant cells, gas exchange, and the regulation of certain hormonal activities, including auxin. This hormonal influence might be the main reason for the observed increase in the number and weight of leaves in this study.

Salicylic acid plays a multifaceted role in regulating various plant functions. It influences stomatal function, chlorophyll content, transpiration and respiration rates, cellular metabolism, ion uptake and transport, membrane permeability, and the synthesis of polyamines like putrescine, spermidine, and spermine (Korver et al., 2018; Perez-Llorca et al., 2019). Additionally, it alters the plant's hormonal balance by increasing auxin and cytokinin levels, ultimately enhancing its biological functions (Shakirova et al., 2003). In our study, a concentration of 450 mg L^{-1} of salicylic acid significantly impacted several growth parameters in Alstroemeria plants. Research suggests that salicylic acid promotes cell

elongation and division through auxin regulation (Korver et al., 2018). This mechanism could explain the observed increase in fresh and dry leaf weight in our experiment. Furthermore, salicylic acid enhances protein synthesis and the production of new isozyme bands, which can lead to flower induction, increased bud formation, and improved flowering (Khorshidi et al., 2019). It also contributes to the plant's photosynthetic system and protection, including RUBISCO activity and the content of photosynthetic pigments (Perez-Llorca et al., 2019). Vlot et al. (2009) proposed that the improved flowering observed after salicylic acid application might be due to its indirect effect on the synthesis or signaling pathways of other plant hormones such as ethylene, auxin, and jasmonic acid.

e) Chlorophyll *a*

Foliar application of calcium chloride and salicylic acid significantly increased chlorophyll *a* content compared to the control (Fig. 6). Among the tested concentrations, the highest chlorophyll *a* content (2.99 mg g^{-1} FW) was observed for plants treated with 450 mg L^{-1} salicylic acid and 225 mg L^{-1} calcium chloride, while the control had the lowest content (0.51 mg g^{-1} FW).

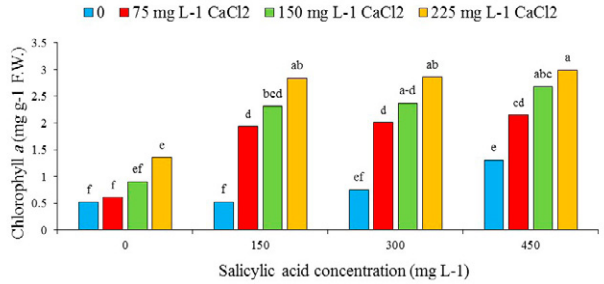

Fig. 6. The effect of various concentrations of calcium chloride and salicylic acid on chlorophyll *a* content of Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test (P≤0.01).

f) Chlorophyll *b*

An analysis of the average values revealed that applying calcium chloride and salicylic acid as a foliar spray significantly increased chlorophyll *b* content compared to the control group. Among the tested concentrations, the combination of 300 or 450 mg L^{-1} salicylic acid with 150 or 225 mg L^{-1} calcium chloride resulted in the highest chlorophyll *b* content, reaching 1.75 mg g^{-1} fresh weight (FW). The control group, which did not receive the foliar spray, had the lowest chlorophyll *b* content, at 0.56 mg g^{-1} FW (Fig. 7).

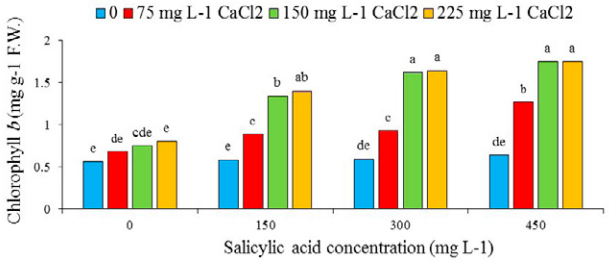

Fig. 7. The effect of various concentrations of calcium chloride and salicylic acid on chlorophyll *b* content of Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test (P≤0.01).

g) Total Chlorophyll

The results showed that all concentrations of calcium chloride and salicylic acid tested led to an increase in total chlorophyll content compared to the control group. The highest total chlorophyll content (4.61 mg g^{-1} fresh weight (FW)) was observed when applying 300 or 450 mg L^{-1} of salicylic acid combined with 150 or 225 mg L^{-1} of calcium chloride. The control treatment, which did not receive the application, had the lowest total chlorophyll content (1.10 mg g^{-1} FW) (Fig. 8).

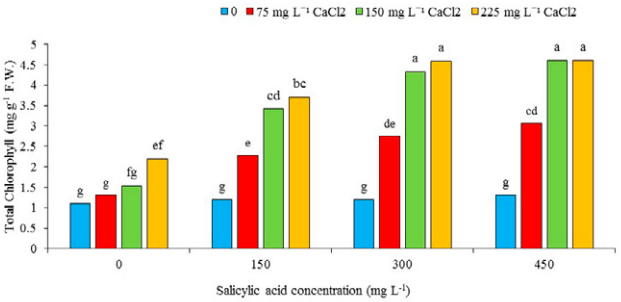

Fig. 8. The effect of various concentrations of calcium chloride and salicylic acid on total chlorophyll content of Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

This current study demonstrates that both calcium chloride and salicylic acid contribute to enhanced levels of photosynthetic pigments, including chlorophyll *a*, *b*, and total chlorophyll. Among the various calcium chloride concentrations tested, 150 and 225 mg L^{-1} yielded the most favorable effects on the photosynthetic pigments of Alstroemeria plants. Calcium plays a critical role in plant health and survival. It facilitates the detoxification of organic acids within vacuoles, which is crucial for plant survival. Additionally, it is essential for both cell division and growth. Furthermore, calcium acts as a key regulator of cellular membrane activity, as documented by Tabatabaie (2014). Beyond this, calcium has been established as a pivotal regulator of numerous physiological and biochemical processes within plants. During the biosynthesis of chlorophyll, light can directly interact with calcium ions, leading to a significant improvement in the aquatic conditions that plants experience throughout their lifecycle. This interaction between light and calcium ions ultimately results in increased photosynthesis and chlorophyll content, as reported by Guo et al. (2021). Our findings align with these previous observations, as Mirzaie Esgandian et al. (2019) demonstrated that foliar application of 2 g L^{-1} nano-chelated calcium to *Gerbera* L. plants significantly increased levels of chlorophyll *a*, *b*, and total chlorophyll.

Salicylic acid acts as a multifaceted regulator in plants, influencing a broad spectrum of processes including stomata function, chlorophyll content, cellular metabolism, ion transport, membrane permeability, and even flower development (Korver et al., 2018; Perez-Llorca et al., 2019). Notably, it enhances photosynthesis by inhibiting chlorophyll degradation. Salicylic acid achieves this by blocking chlorophyll oxidase activity and influencing Abscisic acid function, thereby reducing ethylene production (Wang et al., 2022; Ali et al., 2018). This aligns with findings by Khandan-Mirkohi et al. (2021) who observed increased chlorophyll content in statice cut flowers treated with salicylic acid. Our own results support these observations.

h) Soluble Sugar in Leaves and Petals

Calcium chloride and salicylic acid application significantly increased soluble sugar content in leaves compared to the control group. The highest level (107.94 mg g^{-1} FW) was observed with a combined application of 150 mg L^{-1} calcium chloride and 450 mg L^{-1} salicylic acid, representing a 3.37-fold increase compared to the control (Fig. 9).

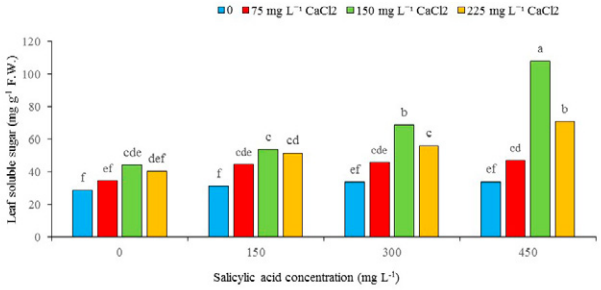

Fig. 9. The effect of various concentrations of calcium chloride and salicylic acid on leaf soluble sugar content of Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

Comparing the mean values, the application of calcium chloride and salicylic acid significantly increased the soluble sugar content in petals compared to the control group. The highest level of soluble sugar in petals (103.63 mg g^{-1} FW) was achieved by applying 225 mg L^{-1} of calcium chloride along with 450 mg L^{-1} of salicylic acid. This value represents a 2.99-fold increase compared to the soluble sugar content in petals of the control treatment (Fig. 10).

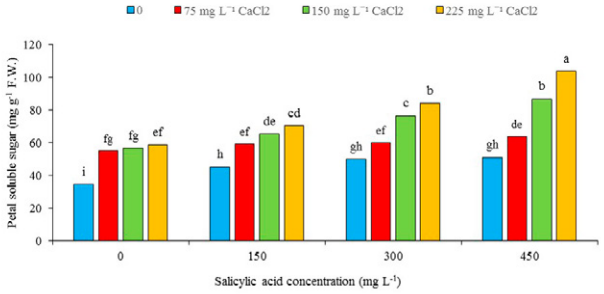

Fig. 10. The effect of various concentrations of calcium chloride and salicylic acid on petal soluble sugar content of Alstroemeria 'Orange Queen'. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

Previous figures demonstrated that calcium chloride treatment increased the total soluble sugar content in both floral organs and leaves compared to the control plants. This rise in soluble sugars likely acts as an osmotic protector, helping plants adjust their internal pressure in response to environmental stresses, as reported by Afzal et al. (2021). Sugars are known to play a stabilizing role in plants, protecting lipids and proteins by preventing unwanted interactions between cell membranes. This stability is achieved through various mechanisms, including hydrogen bonding, gene regulation, and osmotic regulation (Ruamrungsri and Inkham, 2017). In plants, photosynthesis, primarily occurring in leaves, is the main source of sugars used in various cellular processes (Wang et al., 2022). Our study demonstrated that calcium application significantly increased chlorophyll content, particularly at a concentration of 225 mg L^{-1}. Given the close link between chlorophyll content and sugar synthesis, it's unsurprising that the total sugar content also followed an increasing trend. Notably, the 225 mg L^{-1} calcium chloride treatment proved most effective in enhancing both chlorophyll and total sugar content in both leaves and flowers of Alstroemeria plants. This aligns with similar findings reported by Ruamrungsri and Inkham (2017) for *Polianthes tuberosa*.

Our study demonstrated that salicylic acid application improved specific biochemical properties in Alstroemeria plants, including an increase in total soluble sugar content within both leaves and flowers. The most significant effect was observed at a concentration of 450 mg L^{-1} of salicylic acid. We propose that salicylic acid enhances sugar levels in plants through a multi-faceted mechanism: promoting photosynthetic pigments, mitigating oxidative stress, and safeguarding macromolecules like proteins, chloroplasts, and cell membranes. Beyond their primary roles, sugars are known to contribute to osmotic regulation within plants. Notably, salicylic acid appears to delay the degradation of photosynthetic pigments. Additionally, Pasternak et al. (2019) suggest that salicylic acid likely exerts its positive influence on sugar content by preserving the structure and activity of the enzyme RUBISCO. Furthermore, Klessig et al. (2018) reported that salicylic acid application contributes to the preservation and increase of soluble solids by reducing the rate of respiration and altering its underlying mechanisms. This combined effect is likely the primary driver behind the observed increase in soluble sugar content in our findings.

i) Postharvest Traits

i.1) Malondialdehyde

Figure 11 reveals a consistent trend: malondialdehyde (MDA) content decreased with increasing concentrations of calcium chloride (up to 150 mg L^{-1}) and salicylic acid, compared to the control. While MDA content exhibited an increase across all treatments over time, this increase remained statistically insignificant compared to the control. As evidenced in Figure 11, the control group consistently displayed the highest MDA content across all three sampling points.

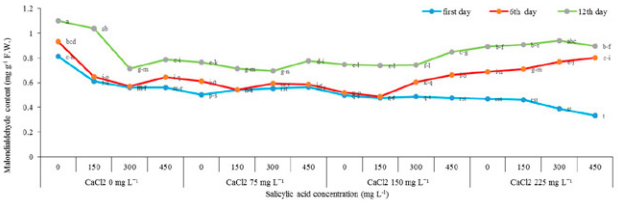

Fig. 11. The effect of various concentrations of calcium chloride and salicylic acid on malondialdehyde content of petals of Alstroemeria 'Orange Queen' during vase life period. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

Figure 11 demonstrates a positive effect of calcium chloride on postharvest quality parameters in Alstroemeria, as evidenced by the reduction in malondialdehyde (MDA) content. During senescence, hydrogen peroxide (H_2O_2) reacts with superoxide anions (O_2^-) to form highly reactive hydroxyl radicals (OH^-). These hydroxyl radicals, in turn, peroxidize membrane lipids by reacting with the methylene groups of unsaturated fatty acids, leading to an increase in MDA content (Gupta et al., 2019). Research by El-Serafy (2019) suggests that during the postharvest period, calcium chloride application can significantly reduce ionic leakage and membrane lipid degradation in rose cut flowers and other horticultural products like cut flowers and fruits. This protective effect is attributed to the enhancement of the plant's antioxidant system, improved photosynthetic efficiency, and reduced reactive oxygen species (ROS) accumulation, ultimately leading to a decrease in membrane lipid peroxidation.

Our findings demonstrate that salicylic acid treatment significantly reduces malondialdehyde (MDA) content in Alstroemeria. This suggests that salicylic acid may prevent membrane degradation and ionic leakage by mitigating the production of reactive oxygen species (ROS), which are the primary culprits behind lipid peroxidation and the consequent increase in MDA content. The reduction in MDA content can also be attributed to salicylic acid's ability to suppress free radical generation. These free radicals contribute to lipid peroxidation, ultimately altering the synthesis of macromolecules in the cell membrane and cytoplasm (Saleem et al., 2021). Supporting our observations, Khandan-Mirkohi et al. (2021) reported a similar decrease in MDA content in salicylic acid-treated statice plants.

i.2) Ascorbate Peroxidase and Catalase Activity

Treatment with different combinations of calcium chloride and salicylic acid concentrations led to a progressive increase in ascorbate peroxidase (APX) activity over time. As depicted in Figure 12, the highest APX activity (1.96 µmol min^{-1} g^{-1} FW) was observed on day 12 when treated with 225 mg L^{-1} calcium chloride and 450 mg L^{-1} salicylic acid, representing a 5.5-fold increase compared to the control. Notably, on day 0, APX activity remained relatively constant across all treatments except the control, suggesting minimal basal activity.

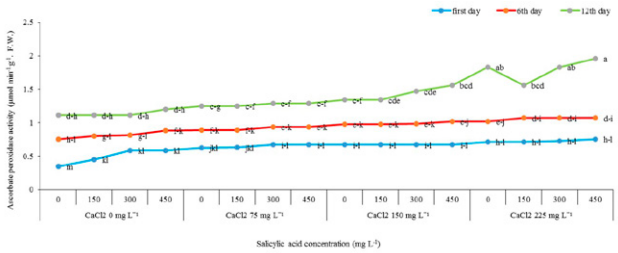

Fig. 12. The effect of various concentrations of calcium chloride and salicylic acid on ascorbate peroxidase activity of petals of Alstroemeria 'Orange Queen' during vase life period. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

Similar to the observed trend with ascorbate peroxidase, foliar application of various calcium chloride and salicylic acid combinations resulted in a progressive increase in catalase activity over time (Fig. 13). The highest catalase activity (1.02 µmol min^{-1} g^{-1} FW) was observed on day 12 when treated with 225 mg L^{-1} calcium chloride and 450 mg L^{-1} salicylic acid, representing a significant increase compared to the control treatment (0.09 µmol min^{-1} g^{-1} FW). Notably, catalase activity remained constant across all treatments on day 0, indicating minimal basal activity.

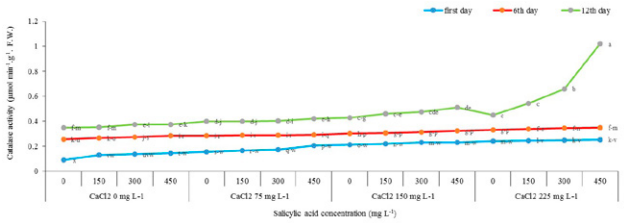

Fig. 13. The effect of various concentrations of calcium chloride and salicylic acid on catalase activity of petals of Alstroemeria 'Orange Queen' during vase life period. Different letters indicate statistically significant differences between the treatments and control according to a Tukey test ($p \leq 0.01$).

Our findings indicate that calcium chloride and salicylic acid effectively enhance the activity of antioxidant enzymes in Alstroemeria, playing a crucial role in mitigating postharvest stress. During this critical period, reactive oxygen species (ROS) levels significantly increase, causing substantial damage to plant cell membranes and antioxidant systems (Nousis et al., 2023). Extensive research has established that plants utilize a combination of enzymatic (ascorbate peroxidase, peroxidase, superoxide dismutase, and catalase) and non-enzymatic systems to eliminate and decompose hydrogen peroxide and other harmful free radicals. As previously discussed, ROS are continuously produced within various plant organs, including chloroplasts, mitochondria, and plasma membranes, throughout their lifecycle. Under normal circumstances, a robust plant defense system effectively prevents excessive ROS accumulation. However, during senescence or degradation, ROS production can surpass this defense, leading to detrimental cellular damage. This phenomenon, known as oxidative stress, manifests as damage to proteins, RNA, DNA, and cell membranes, ultimately culminating in lipid peroxidation (Nousis et al., 2023). Notably, while cut flowers possess intrinsic antioxidant enzymatic systems like superoxide dismutase and catalase, these defenses are often insufficient to adequately mitigate the detrimental effects of free radical-induced oxidation. Therefore, the application of compounds like calcium chloride, which can reinforce the plant's existing antioxidant systems, becomes crucial in enhancing stress-resistance and reducing the detrimental effects of free radicals.

Consistent with the presented figures, salicylic acid treatment demonstrably enhanced the activity of antioxidant enzymes in Alstroemeria. This finding aligns with prior research highlighting salicylic acid's ability to implicitly and explicitly activate antioxidant enzymes, such as peroxidase (Shahmoradi and Naderi, 2018). Notably, Zamani et al. (2011) reported elevated antioxidant activity and decreased hydrogen peroxide and lipid peroxidation upon salicylic acid treatment in rose cut flowers. Our study echoes these findings, demonstrating significant increases in ascorbate peroxidase and catalase activity, as well as enhanced overall antioxidant capacity, following salicylic acid application. Furthermore, salicylic acid treatment was observed to induce the accumulation of phenolic compounds and increase peroxidase enzyme content, suggesting a direct relationship between these two factors. Consequently, this treatment contributes to increased membrane stability, allowing the plant to better withstand adverse conditions. These findings are consistent with previous reports and suggest that salicylic acid application promotes improved Alstroemeria growth by bolstering the antioxidant defense system and mitigating oxidative stress.

Conclusions

In conclusion, the findings of this study suggest that the combined application of salicylic acid and calcium chloride can effectively enhance the postharvest quality and vase life of cut Alstroemeria flowers. The optimal concentrations for these improvements were determined to be 225 mg L^{-1} for calcium chloride and 450 mg L^{-1} for salicylic acid. These findings could be valuable for the commercial floriculture industry, as they provide a potential means to extend the vase life and improve the overall quality of cut Alstroemeria flowers.

Acknowledgments

The authors express their thanks to the Department of Horticultural Sciences, Faculty of Agriculture, Urmia University, who provided the necessary greenhouse and laboratory facilities to conduct this research.

Author Contribution

STA: Performed the experiment, chemical analysis, statistical analysis and preparing the manuscript. ZJ: Assistance in designing the experiment, chemical and statistical analysis and writing the manuscript.

Conflict of Interest

The authors declare that they have no potential conflict of interest in the submitted work.

Data Availability Statement

Data will be made available on request.

References

AEBI, H. Catalase *in vitro*. **Methods in Enzymology**, v.105, p.121-126, 1984. https://doi.org/10.1016/s0076-6879(84)05016-3

AFZAL, S.; CHAUDHARY, N.; SINGH, N.K. **Role of soluble sugars in metabolism and sensing under abiotic stress.** In: AFTAB, T., HAKEEM, K.R. (eds). Plant Growth Regulators. Cham.: Springer, 2021.

ALI, S.; GANAI, B.A.; KAMILI, A.N.; BHAT, A.A.; MIR, Z.A.; BHAT, J.A.; TYAGI, A.; ISLAM S.T.; MUSHTAQ M.; YADAV, P.; RAWAT, S.; GROVER, A. Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. **Microbiological Research**, v.29, p. 212-213. 2018. https://doi.org/10.1016/j.micres.2018.04.008

BARKER, V.A.; PILBEAM, J.D. **Handbook of Plant Nutrition**. Boca Raton: CRC Press, Taylor and Francis Group, 2007.

CAKMAK, I.; HORST, W.J. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). **Physiologia Plantarum**, v.83, n.3, p.463-468, 1991. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

EL-SERAFY, R.S. Silica nanoparticles enhance physio-biochemical characters and postharvest quality of *Rosa hybrida* L. cut flowers. **Journal of Horticultural Research**, v.27, n.1, p.47-54, 2019. https://doi.org/10.2478/johr-2019-0006

GUO, Y.; LIU, Y.; ZHANG, Y.; LIU, J.; GUL, Z.; GUO, X.-R.; ABOZEID, A.; TANG, Z.-H. Effects of exogenous calcium on adaptive growth, photosynthesis, ion homeostasis and phenolics of *Gleditsia sinensis* Lam. plants under salt stress. **Agriculture**, v.11, p.978, 2021. https://doi.org/10.3390/agriculture11100978

GUPTA, D.K.; PALMA, J.M. CORPAS, F.J. **Nitric oxide and hydrogen peroxide signaling in higher plants**. Berlin: Springer, 2019. 275p.

IRIGOYEN, J.J.; EMERICH, D.W.; SANCHEZ-DIAZ, M. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. **Physiologia Plantarum**, v.84, p.55-60, 1992. https://doi.org/10.1111/j.1399-3054.1992.tb08764.x

KANG, H.M.; SALTVEIT, M.E. Chilling tolerance of maize, cucumber and rice seedling leaves and roots and differentially affected by salicylic acid. **Plant Physiology**, v.115, p.571-576, 2002. https://doi.org/10.1034/j.1399-3054.2002.1150411.x

KHANDAN-MIRKOHI, A.; PIRGAZI, R.; TAHERI, M.R.; AJDANIAN, L.; BABAEI, M.; JOZAY, M.; HESARI, M. Effects of salicylic acid and humic material preharvest treatments on postharvest physiological properties of statice cut flowers. **Scientia Horticulturae**, v. 283, p.110009, 2021. https://doi.org/10.1016/j.scienta.2021.110009

KHOLDEBARIN, B.; ESLAMZADEH, T. **Mineral nutrition of plants** (Vol. 1). Shiraz: Shiraz University Pub., 2001. pp.358-360. (in Persian)

KHORSHIDI, R.; JOKAR, A.; GOLMAKANI, M.T. The effect of foliar spraying of salicylic acid and potassium sulfate on quality and quantity of *Pelargonium graveolens*. **Journal of Plant Research (Iranian Journal of Biology)**, v.32, n.1, p.1-8, 2019. (in Persian)

KLESSIG, D.F.; CHOI, H.W.; DEMPSEY, D.A. Systemic acquired resistance and salicylic acid: past, present, and future. **Molecular Plant-Microbe Interactions**, v.31, p.871-888, 2018. https://doi.org/10.1094/MPMI-03-18-0067-CR

KORVER, R.A.; KOEVOETS, I.T.; TESTERINK, C. Out of shape during stress: a key role for auxin. **Trends in Plant Science**, v.23, p.783-793, 2018. https://doi.org/10.1016/j.tplants.2018.05.011

LICHTENTHALER, H.K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. **Methods in Enzymology**, v.148, p.350-382, 1987. https://doi.org/10.1016/0076-6879(87)48036-1

LUO, Y.; LIU, M.; CAO, J.; CAO, F.; ZHANG, L. The role of salicylic acid in plant flower development. **Forestry Research**, v.2, p.14-20, 2022. https://doi.org/10.48130/FR-2022-0014

MADY, E.; ABD El-WAHED, A.H.M.; AWAD, A.H.; ASAR, T.O.; AL-FARGA, A.; ABD EL-RAOUF, H.S.; RANDHIR, R.; ALNUZAILI, E.S.; EL-TAHER, A.M.; RANDHIR, T.O.; HAMADA, F.A. Evaluation of salicylic acid effects on growth, biochemical, yield, and anatomical characteristics of eggplant (*Solanum melongena* L.) plants under salt stress conditions. **Agronomy**, v.13, n.9, p.2213, 2023. https://doi.org/10.3390/agronomy13092213

MIRZAIE ESGANDIAN, N.; JABBARZADEH, Z.; RASOULI-SADAGHIANI, M.H. Investigation of some morphological and biochemical characteristics and vase life of *Gerbera jamesonii* cv. Dune cut flower using humic acid and nano calcium chelate. **Iranian Journal of Horticultural Science and Technology**, v.20, n.2, p.157-170, 2019. (in Persian)

MOALLAYE MAZAEI, S.; CHEHRAZI, M.; KHALEGHI, E. The effect of calcium nanochelate on morphological, physiological, biochemical characteristics and vase life of three cultivars of gerbera under hydroponic system. **Plant Productions**, v.43, n.1, p.53-66, 2020. (in Persian)

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. **Plant Cell Physiology**, v.22, p.867-880, 1981. https://doi.org/10.1093/oxfordjournals.pcp.a076232

NASERI, M.T.; EBRAHIMI GERAVI, M. **Physiology of bulbous plants**. Mashhad: Jahad Daneshgahi of Mashhad press, 2002. 352p. (in Persian)

NAZARI, F. The effect of foliar application of calcium chloride and nanocalcium chelated on vegetative, reproductive and post-harvest life of tuberose (*Polianthes tuberosa* L.). **Journal of Plant Research**, v.32, n.2, p.1-14, 2019. (in Persian)

NOUSIS, L.; KANAVAROS, P.; BARBOUTI, A. Oxidative stress-induced cellular senescence: is labile iron the connecting link? **Antioxidants**, v.12, n.6, p.1250, 2023. https://doi.org/10.3390/antiox12061250

PASTERNAK, T.; GROOT, E.P.; KAZANTSEV, F.V.; TEALE, W.; OMELYANCHUK, N.; KOVRIZHNYKH, V.; PALME, K.; MIRONOVA, V.V. Salicylic acid affects root meristem patterning via auxin distribution in a concentration-dependent manner. **Plant Physiology**, v.180, p.1725-1739, 2019. https://doi.org/10.1104/pp.19.00130

PEREZ-LLORCA, M.; MUNOZ, P.; MULLER, M.; MUNNE-BOSCH, S. Biosynthesis, metabolism and function of auxin, salicylic acid and melatonin in climacteric and non-climacteric fruits. **Frontiers in Plant Science**, v.10, n.136, p.1-10, 2019. https://doi.org/10.3389/fpls.2019.00136

REDDY, A.R.G.; SARKAR, M.M. **Effect of calcium on Gladiolus**. Charlottesville: Lambert Press, 2016. 60p.

RUAMRUNGSRI, S.; INKHAM, C. Effect of calcium nitrate addition on growth and bulb quality of *Hippeastrum*. **Acta Horticulturae**, v.54, n.1167, p.375-380, 2017. https://doi.org/10.17660/ActaHortic.2017.1167.54

SALEEM, M.; FARIDUDDIN, Q.; CASTROVERDE, C.D.M. Salicylic acid: A key regulator of redox signaling and plant immunity. **Plant Physiology and Biochemistry**, v.168, p.381-397, 2021. https://doi.org/10.1016/j.plaphy.2021.10.011

SHAHMORADI, H.; NADERI, D. Improving effects of salicylic acid on morphological, physiological and biochemical responses of salt-imposed winter jasmine. **International Journal of Horticultural Science and Technology**, v.5, n.2, p.219-230, 2018. https://doi.org/10.22059/ijhst.2018.259507.246

SHAKIROVA, M.F.; SAKHABUTDINOVA, A.R.; BEZRUKOVA, M.V.; FATKHUTDINOVA, R.A.; FATKHUTDINOVA, D.R. Change in the hormonal status of wheat seedling induced by salicylic acid and salinity. **Journal of Plant Science**, v.164, n.3, p.317-322, 2003. https://doi.org/10.1016/S0168-9452(02)00415-6

TABATABAIE, J. **Principles of mineral nutrition of plants**. Tabriz: Tabriz University Press, 2014. 652p. (in Persian)

TOFIGHI ALIKHANI, T.; TABATABAEI, S.J.; MOHAMMADI TORKASHVAND, A.; TALEI, D. Silica nanoparticles and calcium on the histological characteristics and stem bending in gerbera cut flower. **Ornamental Horticulture**, v.27, n.3, p.334-343, 2021. https://doi.org/10.1590/2447-536X.v27i3.2308

VLOT, A.C.; DEMPSEY, M.A.; KLESSIG, D.F. Salicylic acid, a multifaceted hormone to combat disease. **Annual Reviews of Phytopathology**, v.47, p.177-206, 2009. https://doi.org/10.1146/annurev.phyto.050908.135202

WANG, K.; SHENG, Y.; WANG, H.; HE, S.; KIM, W.S.; SHANG, W.; WANG, Z.; SHI, L. Effects of exogenous salicylic acid (SA), 6-benzylaminopurine (6-BA), or abscisic acid (ABA) on the physiology of *Rosa hybrida* 'Carolla' under high-temperature stress. **Horticulturae**, v.8, n.9, p.851, 2022. https://doi.org/10.3390/horticulturae8090851

ZAMANI, S.; KAZEMI, M.; ARAN, A. Postharvest life o Rose flowers as affected by salicylic acid and glutamine. **World Applied Science Journal**, v.12, n.9, p.1621-1624, 2011.