

Calcium Nitrate Foliar Spray Enhances Photosynthesis and Antioxidant Activity, Improving Rose Vase Life

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Increasing photosynthesis is crucial for enhancing growth, also boosting antioxidant activity is essential for improving the quality and vase life of roses. This study evaluates how foliar application of calcium nitrate ($\text{Ca}(\text{NO}_3)_2$) at different concentrations affects photosynthesis, antioxidant enzyme activity, and vase life in roses to achieve this objective. In a factorial experiment based on a randomized complete design, the 'Samurai' and 'Jumilia' rose cultivars were subjected weekly to three concentrations of $\text{Ca}(\text{NO}_3)_2$: 0 mg/L (control), 80 mg/L, and 160 mg/L. The results showed that a 160 mg/L $\text{Ca}(\text{NO}_3)_2$ improved photosynthesis by 23.3% in 'Jumilia' and 35.3% in 'Samurai' providing evidence of enhanced growth traits in both rose cultivars. Additionally, the application of 160 mg/L $\text{Ca}(\text{NO}_3)_2$ increased transpiration, which subsequently led to higher calcium concentrations in the leaves and petals of 'Samurai' (by 2.9- and 2.5-fold, respectively) and 'Jumilia' (by 1.9- and 1.6-fold, respectively). The use of 160 mg/L $\text{Ca}(\text{NO}_3)_2$ elevated the activities of peroxidase, superoxide dismutase, and catalase, leading to a 10.4% and 13.6% increase in the membrane stability index in 'Samurai' and 'Jumilia', respectively, which resulted in an increase in vase life by 4.33 and 4.03 days for 'Samurai' and 'Jumilia', respectively. Overall, applying 160 mg/L $\text{Ca}(\text{NO}_3)_2$ enhanced flower quality and boosted antioxidant enzyme activity, resulting in prolonged vase life for both rose cultivars.

Abstract

Keywords: Calcium nutrition, Growth, Membrane integrity, Photosynthesis, Postharvest quality.

INTRODUCTION

Rosa hybrida L. ranks among the top-selling and most significant commercial products in the international cut flower market (Darras, 2021). The longevity of rose flowers is a critical factor affecting their quality and marketability. Significant research efforts have been dedicated to enhancing the vase life and overall quality of roses (Banijamali *et al.*, 2018). If pre-harvest factors, including nutrients, are properly managed, they have a significant impact on the vase life of cut flowers (Verdonk *et al.*, 2023).

Among the macronutrients present in nutrient solutions, calcium (Ca) plays a pivotal role in improving the quality characteristics of cut roses (Khosravi *et al.*, 2024). It is essential for maintaining cell wall structure by forming Ca pectate cross-links in the middle lamella, which strengthens tissue integrity (Huang *et al.*, 2023). Additionally, Ca contributes to membrane stability by binding to phospholipids and proteins, reducing ion leakage and preserving cellular functions under stress conditions (Huang *et al.*, 2023; Khalili *et al.*, 2023). Furthermore, foliar-applied Ca can enhance antioxidant enzyme activity, reducing oxidative stress and delaying flower senescence (Sairam *et al.*, 2011).

Low-transpiration organs, such as flowers, often experience Ca deficiencies due to limited translocation, emphasizing the importance of targeted Ca application (Huang *et al.*, 2023). Given its immobility within plants, consistent Ca provision is required to support leaf and root growth, which can be achieved through foliar application (Amor and Marcelis, 2003; Youssef *et al.*, 2017). Previous studies have also reported the impact of pre-harvest foliar application of calcium on rose floral characteristic (Banijamali *et al.*, 2018; Khosravi *et al.*, 2024). Foliar-applied calcium can penetrate leaf surfaces as a passive process driven by the concentration gradient between the leaf surface and interior (Eichert and Fernández, 2023). Bahamonde *et al.* (2018) demonstrated that calcium is preferentially absorbed by veins in beech leaves, where the cuticle above the veins differs in structure and composition from the surrounding cuticle. This unique structural property facilitates calcium absorption into vascular tissues. Although calcium uptake into the leaf cells is a separate process, this mechanism supports the targeted delivery of calcium to critical plant tissues, potentially reaching low-transpiration organs like petals under specific conditions.

Considering the significant metabolic role of Ca in plants, an adequate and timely provision of Ca during the growth and post-harvest stages improves longevity and nutritional quality of plant products. In this study, $\text{Ca}(\text{NO}_3)_2$ was chosen over other forms of calcium due to its dual provision of Ca and nitrate, which supports both structural integrity and metabolic activities in plants. Unlike calcium chloride, calcium nitrate reduces the risk of chloride toxicity and enhances nitrogen assimilation. Additionally, its high solubility ensures efficient uptake and distribution within the plant, making it highly suitable for foliar applications. In fact, according to previous research, during cultivation, $\text{Ca}(\text{NO}_3)_2$ is the preferred compound for many fertilizers and standard nutritional formulations as it is a water-soluble source of calcium (Wu *et al.*, 2023).

The ‘Jumilia’ and ‘Samurai’ rose cultivars were selected for this study based on their commercial significance and contrasting phenotypic traits, which offer insights into the generalizability of the findings. ‘Samurai’, with its robust growth characteristics, and Jumilia, valued for its aesthetic qualities, represent a spectrum of responses to pre-harvest treatments.

This study aims to assess the photosynthetic activity of plants during the vegetative growth period and to examine the antioxidant enzyme activity of cut flowers under foliar application of $\text{Ca}(\text{NO}_3)_2$. By exploring the interaction between these factors, the findings provide valuable insights into maximizing the vase life and quality of roses.

MATERIALS AND METHODS

Plant materials and treatments

The study involved two rose cultivars (*Rosa hybrida* L.), ‘Jumilia’ and ‘Samurai’ (Fig. 1), grown hydroponically in 100% perlite substrate. The physical and chemical properties of these substrates are listed in table 1. To ensure uniform plant growth, a half-strength modified Hoagland nutrient solution (Table 2) was provided for 30 days, transitioning to a full-strength Hoagland solution until the experiment was concluded.

Forty-five days after planting, the plants were topped, and one week later, foliar sprays of calcium nitrate were applied weekly for six months. Foliar application of $\text{Ca}(\text{NO}_3)_2$ solutions was performed at concentrations of 0 mg/L (distilled water), 80 mg/L, and 160 mg/L.

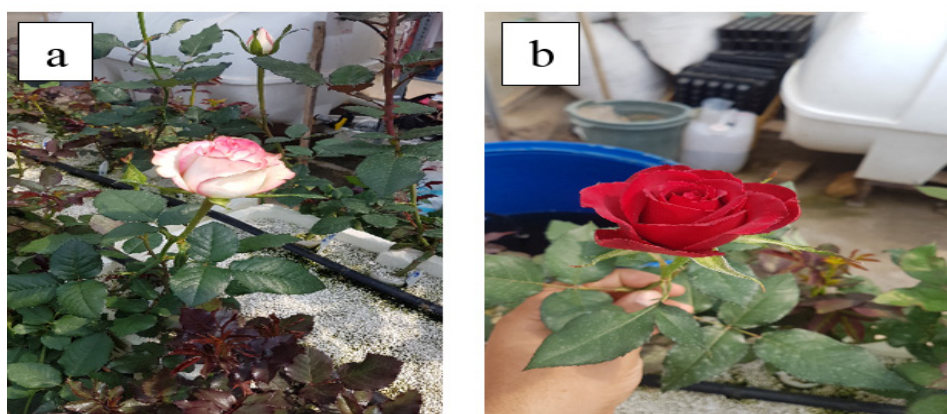


Fig. 1. The two rose cultivars used in the study: (a) ‘Jumilia’ and (b) ‘Samurai’.

Table 1. Physical and chemical properties of media used.

Water holding (%)	Bulk density (g/cm ³)	Total porosity (%)	Air capacity (%)	EC (dS/m)	pH
69.8	0.13	68.0	15	1.60	7.80

Table 2. The nutritional program used, prepared according to the Hoagland formulation formula.

Compounds		Final concentration of element (ppm)	Element	Volume of stock solution per liter of final solution (ml)	Concentration of stock solution (g/L)
Macro nutrients				N	224
	KNO_3	101.10	6	K	235
	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	236.16	4	Ca	160
	$\text{NH}_4\text{H}_2\text{PO}_4$	115.08	2	P	62
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	246.49	1	S	32
Micro nutrients				Mg	24
	KCl	1.864	2	Cl	1.77
	H_3BO_3	0.773	2	B	0.27
	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.169	2	Mn	0.11
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.288	2	Zn	0.13
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.062	2	Cu	0.03
	H_2MOO_4 (85% MOO_3)	0.040	2	Mo	0.05
	NaFeDTPA (10% Fe)	30.0	0.3-1	Fe	1-3

Experimental setup and data collection

The experiment utilized a completely randomized factorial design, comprising two factors: rose cultivar and calcium nitrate concentration. Each treatment was replicated four times, with each replication consisting of six plants (one plant per observation). Data presented in this study represent the mean of four replicates $\pm$ standard error (SE).

Irrigation and greenhouse conditions

Plants were cultivated using an open hydroponic system with drip irrigation. Irrigation cycles lasted 2–4 minutes, delivering 35–40 ml/min per dripper, with eight cycles daily. Seasonal irrigation volumes were adjusted to 1200 ml/day in summer and 600 ml/day in winter. The greenhouse was maintained at $25/16 \pm 2$ °C day/night temperatures, with a relative humidity of 65% and midday light intensity of 240 ± 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD).

Measured parameters and methods

Floral characteristics (flower diameter and length, pedicel length, rosehip diameter, and stem diameter) were measured using a digital caliper. Stem length was assessed with a ruler. Leaf count was performed visually, and leaf area was determined using a leaf area meter (WinArea_UT_10).

Photosynthesis and Transpiration Rates: Measurements were taken on three fully expanded leaves per replicate using a portable photosynthesis system (Li-Cor, Li-6400, USA).

Leaf and Petal Ca Concentration: At the time of harvest, leaf and petal samples were collected and dried at 72 °C for 48 hours, ground, and ashed at 550 °C for 6 hours. The ash was dissolved in 2 M HCl, diluted to 100 ml with distilled water, and analyzed for calcium concentration using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES, Varian 730-ES, Australia) (Volpin and Elad, 1991).

Anthocyanin Concentration in Petals: Five days post-harvest, petal samples (1 g) were extracted with methanol, stored overnight at 4 °C, centrifuged (3000 g, 10 minutes), and analyzed at 550 nm using a spectrophotometer (Shimadzu UV106A) (Wagner, 1979).

Relative water content (RWC): RWC was calculated five days after harvest using the following formula:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where FW is fresh weight, TW is turgid weight, and DW is dry weight (Ghoulam *et al.*, 2002).

Membrane stability index (MSI): MSI was determined five days after harvest using petal samples, as described by Singh *et al.* (2008).

$$\text{MSI (\%)} = \left[1 - \frac{\text{EC1}}{\text{EC2}} \right] \times 100$$

Where EC1 is initial electrical conductivity (40 °C, 30 minutes) and EC2 is final conductivity (100 °C, 15 minutes).

The activity of antioxidant enzymes: Five days after harvest, samples of leaves (0.2 g each) from every replicate and treatment were crushed using liquid nitrogen and combined with a solution containing sodium phosphate, ethylenediaminetetraacetic acid (EDTA), and polyvinylpyrrolidone (PVP). The mixture was centrifuged to obtain a liquid extract for

determining antioxidant enzyme activity. To measure superoxide dismutase (SOD) activity the enzyme extract was mixed with phosphate buffer, riboflavin, nitro blue tetrazolium and methionine. Changes in absorption at 560 nm were recorded employing a spectrophotometer (Shimadzu UV106A). Catalase (CAT) activity was evaluated by combining the enzyme extract with phosphate buffer and hydrogen peroxide, and monitoring the decrease in absorbance at 240 nm. Peroxidase (POD) activity was determined by mixing the enzyme extract with o-dianisidine, and potassium phosphate buffer with absorbance readings taken at 470 nm (Chen *et al.*, 2009). The results, for POD and CAT were represented as $\mu\text{mol min}^{-1} \text{mg}^{-1}$ of total protein while SOD findings were expressed as U mg^{-1} of protein.

Percentage changes in fresh weight: Flower stem weight was initially measured right after harvesting and then on days 2, 4, 6, 8, and 10. Finally, the percentage changes in fresh weight were documented (Torre *et al.*, 1999).

Vase life: To assess vase life, flower stems from every replicate and treatment were cut underwater to 2 cm, with the lower 10 cm of leaves removed to prevent air embolism. Each stem was placed in a container with 500 ml of distilled water, changed every two days. Flowers were maintained at $25 \pm 2^\circ\text{C}$, $65 \pm 5\%$ relative humidity, and 12-hour photoperiods with $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity. Vase life was recorded as the number of days from harvest until visible signs of wilting, defined by petal yellowing, petal dropping, or a bent-neck appearance (greater than 45°).

Statistical analysis

Data were analyzed using SAS software (v9.4). Means were compared using the least significant difference (LSD) test at a 5% significance level.

RESULTS

Growth parameters

Applying $\text{Ca}(\text{NO}_3)_2$ solution significantly increased the length and diameter of the stems (Table 3), although, there was no notable difference between the 160 mg/L and 80 mg/L treatments. Applying $\text{Ca}(\text{NO}_3)_2$ at 160 mg/L concentration increased the length and diameter of the 'Samurai' stem by 16.8% and 19.1%, respectively, and of the 'Jumilia' stem by 38.2% and 29.7%, respectively, compared to the control treatment for both cultivars (Table 3). Spraying of $\text{Ca}(\text{NO}_3)_2$ affected pedicel diameter and length in both rose cultivars (Table 3). Foliar application of 160 mg/L $\text{Ca}(\text{NO}_3)_2$ resulted in an rise in the diameter and length of the pedicel in 'Jumilia' by 16.1% and 14.3%, respectively, and in 'Samurai' by 25% and 15.4%, respectively, compared to the control plants specific to each cultivar (Table 3). Treatment with $\text{Ca}(\text{NO}_3)_2$ resulted in a notable rise in the diameter and length of rose rosehips (Table 3). There was significantly difference between 80 mg/L and 160 mg/L $\text{Ca}(\text{NO}_3)_2$ concentrations in the effect on diameter and length of rose rosehips. The application of $\text{Ca}(\text{NO}_3)_2$ at a concentration of 160 mg/L, compared to the control, resulted in an increase of 17.5% and 20.3% in the diameter and length of the rosehip in 'Jumilia', respectively (Table 3). The maximum diameter and length of the flower were achieved by applying $\text{Ca}(\text{NO}_3)_2$ at 160 mg/L in the 'Jumilia' cultivar (Table 3). The control plants of 'Jumilia' exhibited the smallest flower diameter (30.9 mm), while the control treatment of 'Samurai' had the shortest flower length (38.49 mm) (Table 3). Applying $\text{Ca}(\text{NO}_3)_2$ to the leaves increased the leaf count, though it did not significantly affect the leaf surface area of rose flowers (Table 4). Compared to the untreated group, 'Samurai' showed an 8.1% rise in leaf count with 160 mg/L $\text{Ca}(\text{NO}_3)_2$ treatment. Similarly, 'Jumilia' exhibited a 22% increase in leaf count with 80 mg/L $\text{Ca}(\text{NO}_3)_2$ treatment compared to the control (Table 4).

Table 3. Effects of foliar spray application with various concentrations of $\text{Ca}(\text{NO}_3)_2$ solution on the growth parameters of two rose cultivars studied.

Cultivar	$\text{Ca}(\text{NO}_3)_2$ (mg/L)	Stem length (cm)	Stem diameter (mm)	Pedicle diameter (mm)	Rosehip diameter (mm)
Samurai	0	69.6 ± 1.4c	5.02 ± 0.14d	5.60 ± 0.08d	11.57 ± 0.28b
	80	76.6 ± 3.3b	5.71 ± 0.19b	6.40 ± 0.62c	12.76 ± 0.53a
	160	81.4 ± 3.6a	5.98 ± 0.19a	7.03 ± 0.20b	12.92 ± 0.27a
Jumilia	0	42.1 ± 1.1e	4.31 ± 0.09e	5.20 ± 0.12d	10.87 ± 0.12c
	80	56.1 ± 2.5d	5.36 ± 0.13c	7.36 ± 0.40b	11.57 ± 0.24b
	160	58.2 ± 1.1d	5.59 ± 0.17bc	8.41 ± 0.24a	12.77 ± 0.11a

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Table 3. Continued

Cultivar	$\text{Ca}(\text{NO}_3)_2$ (mg/L)	Flower diameter (mm)	Flower length (mm)	Rose hip length (mm)	Pedicle length (mm)
Samurai	0	31.05 ± 0.82c	38.49 ± 1.30d	12.88 ± 0.20c	11.13 ± 0.19d
	80	33.56 ± 0.56b	40.02 ± 0.79bc	14.27 ± 0.74ab	12.42 ± 0.52ab
	160	34.60 ± 1.88b	40.23 ± 1.14b	14.83 ± 0.38a	12.84 ± 0.17a
Jumilia	0	30.90 ± 0.52c	38.81 ± 0.37cd	11.36 ± 0.52d	10.73 ± 0.32d
	80	31.08 ± 0.77c	41.66 ± 0.37a	12.06 ± 0.34d	11.71 ± 0.35c
	160	36.89 ± 1.17a	42.73 ± 0.41a	13.67 ± 0.49bc	12.26 ± 0.04b

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.Table 4. Effects of foliar spraying with different concentrations of $\text{Ca}(\text{NO}_3)_2$ solution on leaf characteristics of various rose cultivars.

Cultivar	$\text{Ca}(\text{NO}_3)_2$ (mg/L)	Leaf number	Leaf surface area (cm^2)
Samurai	0	14.1 ± 0.52b	724.8 ± 23.8ab
	80	14.2 ± 0.25b	743.0 ± 25.7ab
	160	15.3 ± 0.31a	835.6 ± 231.5a
Jumilia	0	10.7 ± 0.50d	455.2 ± 5.8c
	80	13.1 ± 0.29c	638.8 ± 21.1b
	160	13.0 ± 0.22c	677.8 ± 56.1ab

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Photosynthesis and transpiration rates

Foliar application of $\text{Ca}(\text{NO}_3)_2$ at a concentration of 160 mg/L in the 'Jumilia' and 'Samurai' cultivars resulted in an increase of 23.3% and 35.3% photosynthesis, respectively, compared to the control (Fig. 2a). The lowest level of photosynthesis occurred in the control treatment of 'Samurai' (Fig. 2a). The application of $\text{Ca}(\text{NO}_3)_2$ resulted in an increased transpiration rate in both rose cultivars (Fig. 2b). The 'Jumilia' cultivar showed the highest transpiration rate ($9.37 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) when treated with a 160 mg/L concentration of $\text{Ca}(\text{NO}_3)_2$ via foliar spraying (Fig. 2b). The lowest transpiration rate ($5.89 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was observed in the control plants 'Samurai' (Fig. 2b).

Anthocyanins

The highest amount of anthocyanins was related to the 'Samurai' cultivar; however, $\text{Ca}(\text{NO}_3)_2$ treatment was ineffective (Fig. 2c). The treatment with 160 mg/L $\text{Ca}(\text{NO}_3)_2$ had a

notable impact on the enhancement of anthocyanins in ‘Jumilia’, resulting in an 11.7% increase compared to the control (Fig. 2c).

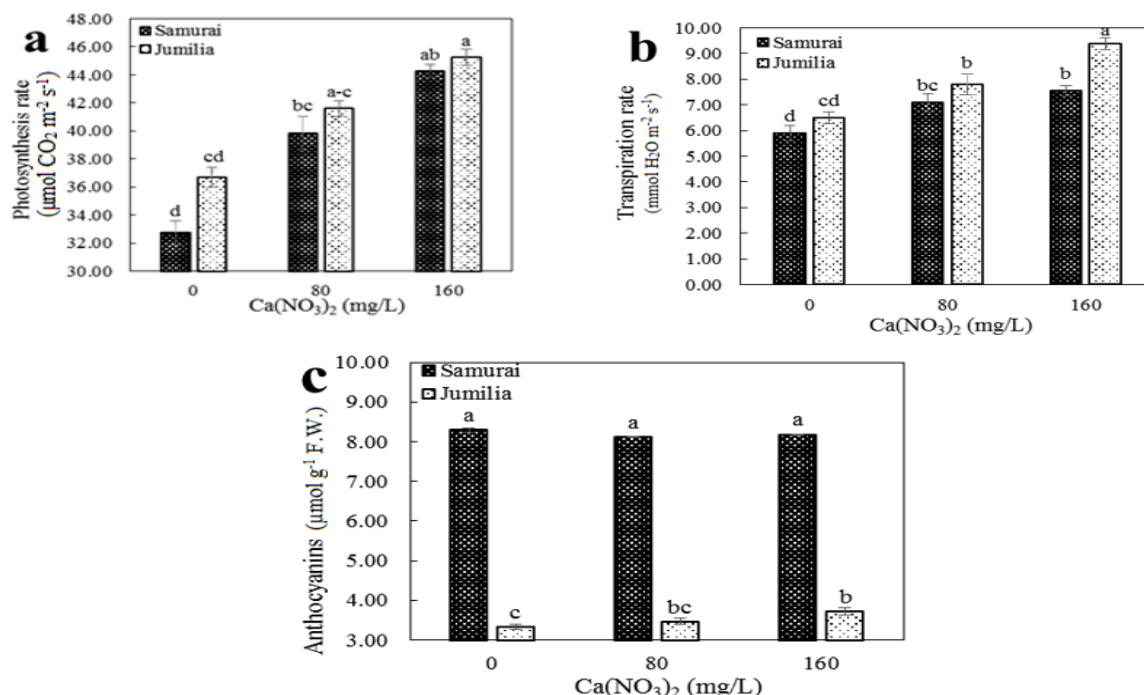


Fig. 2. Effects of foliar spray with concentrations of 0, 80, and 160 mg/L Ca(NO₃)₂ on the photosynthesis (a), transpiration rates (b), and anthocyanins (c) in the leaves of ‘Jumilia’ and ‘Samurai’ cultivars.

Leaf and petal Ca

Ca(NO₃)₂ caused a significant increase in leaf and petal Ca in both rose cultivars, and the higher the concentration of Ca(NO₃)₂, the more pronounced this increase (Table 5). The highest increase was related to the application of 160 mg/L Ca(NO₃)₂ in ‘Samurai’, which, compared to the control, leading to a 2.9 and 2.5-fold raise in leaf and petal Ca, respectively (Table 5). Furthermore, treatment with 160 mg/L Ca(NO₃)₂ in the cultivar ‘Jumilia’ led to 1.9- and 1.6-fold increases in leaf and petal Ca concentrations, respectively, compared to the control. The minimum Ca concentration in both leaves and petals was associated with the control treatment in the ‘Samurai’ cultivar (Table 5).

Table 5. Effects of foliar spraying with different concentrations of Ca(NO₃)₂ solution on leaf and petal Ca of various rose cultivars.

Cultivar	Ca(NO ₃) ₂ (mg/L)	Leaf Ca (ppm)	Petal Ca (ppm)
Samurai	0	7382.5 ± 499.6e	782.1 ± 78.6e
	80	18428.0 ± 498.7b	1724.5 ± 118.5b
	160	21454.7 ± 702.5a	1973.6 ± 103.2a
Jumilia	0	10118.2 ± 784.0d	984.2 ± 97.8d
	80	14227.0 ± 766.4c	1461.7 ± 102.6c
	160	18763.0 ± 847.0b	1623.8 ± 102.2bc

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Relative water content (RWC)

The RWC was noticeably influenced by the application of Ca(NO₃)₂ (Fig. 3a). Applying 80 mg/L Ca(NO₃)₂ via foliar spray increased the RWC by 11.4% in ‘Jumilia’ compared to the control.

In ‘Samurai’, treating with 160 mg/L $\text{Ca}(\text{NO}_3)_2$ resulted in the highest RWC of the leaves, showing an 8% increase compared to the control (Fig. 3a).

Membrane stability index

As shown in fig. 1b, as the concentration of $\text{Ca}(\text{NO}_3)_2$ increased, MSI improved (Fig. 3b). The highest MSI was obtained in ‘Jumilia’ with a foliar application of 160 mg/L $\text{Ca}(\text{NO}_3)_2$, showing a 13.6% increase compared to the control. In ‘Samurai’, plants treated with 160 mg/L $\text{Ca}(\text{NO}_3)_2$ showed a 10.4% increase in the MSI compared to the control (Fig. 3b).

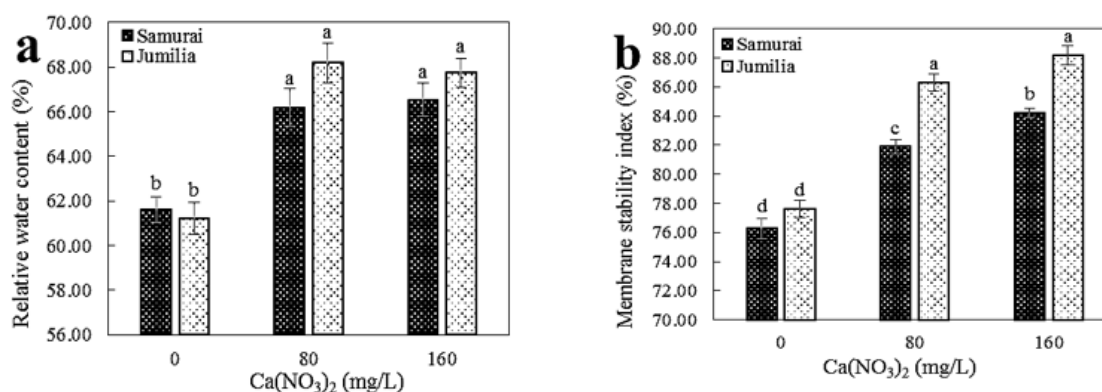


Fig. 3. The impact of foliar spray with concentrations of 0, 80, and 160 mg/L $\text{Ca}(\text{NO}_3)_2$ on the relative water content (a), and membrane stability index (b) in the leaves of ‘Jumilia’ and ‘Samurai’ cultivars. In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

The activity of antioxidant enzymes

Applying $\text{Ca}(\text{NO}_3)_2$ to the leaves significantly influenced the activity of antioxidant enzymes in both cultivars (Fig. 4a, b, and c). Increasing the concentration of $\text{Ca}(\text{NO}_3)_2$ led to a significant rise in POD activity, reaching its peak at a concentration of 160 mg/L (Fig. 4a). In comparison to the control treatment, this led to a respective 43.1% and 80.2% increase in POD enzyme activity in ‘Samurai’ and ‘Jumilia’ (Fig. 4a). The control treatment of ‘Jumilia’ exhibited the lowest POD enzyme activity, measured at $27.29 \mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ (Fig. 4a).

The highest SOD enzyme activity in roses was observed with the use of $\text{Ca}(\text{NO}_3)_2$ concentrations of 80 mg/L and 160 mg/L, which did not show a significant difference between them (Fig. 4b). Applying 160 mg/L $\text{Ca}(\text{NO}_3)_2$ to ‘Samurai’ led to a 56% rise in SOD activity compared to the control (Fig. 4b). In ‘Jumilia’, the greatest SOD enzyme activity was observed with an 80 mg/L $\text{Ca}(\text{NO}_3)_2$ application, resulting in a significant 60.8% increase compared to the control (Fig. 4b). The least activity of the SOD enzyme ($1.30 \text{ U mg}^{-1} \text{protein}$) was related to the control treatment of ‘Jumilia’ (Fig. 4b). Foliar application of $\text{Ca}(\text{NO}_3)_2$ at a concentration of 80 mg/L resulted in a respective increase of 78.7% and 34.4% in CAT enzyme activity in ‘Samurai’ and ‘Jumilia’ compared to the control (Fig. 4c).

Vase life

Spraying $\text{Ca}(\text{NO}_3)_2$ prolonged the longevity of rose flowers (Fig. 4d). The most significant improvement in vase life after harvest occurred with the treatment of 160 mg/L $\text{Ca}(\text{NO}_3)_2$ in ‘Samurai’, where the vase life increased by 4.33 days compared to the control. However, the longest vase life flowers lasted in ‘Jumilia’ was attributed to the use of 80 mg/L $\text{Ca}(\text{NO}_3)_2$ treatment, achieving 15.11 days. The minimum vase life (9.28 days) was associated with the control treatment of ‘Samurai’ (Fig. 4d).

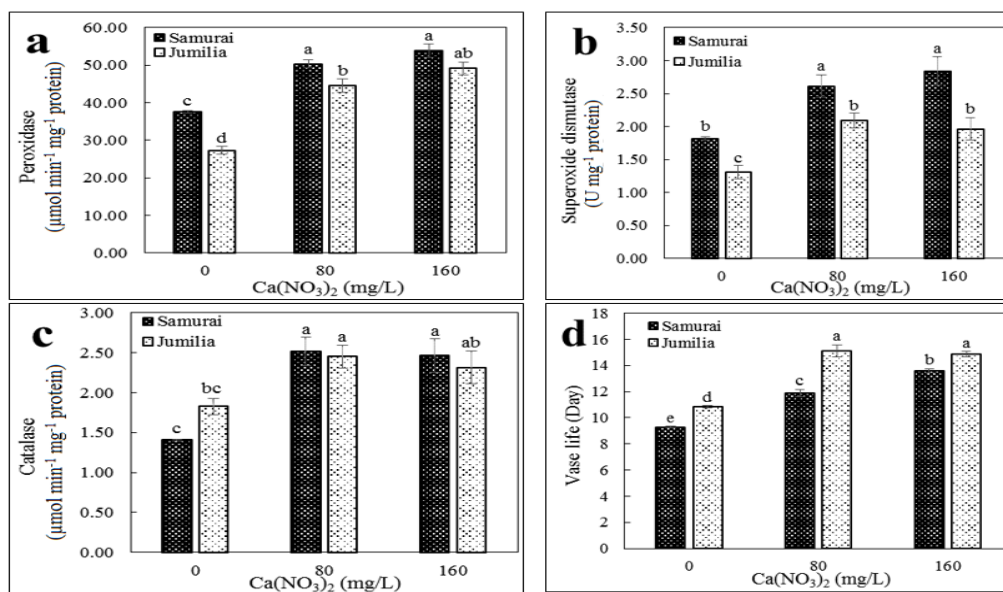


Fig. 4. Effects of foliar spray with concentrations of 0, 80, and 160 mg/L $\text{Ca}(\text{NO}_3)_2$ on peroxidase (a), superoxide dismutase (b), catalase (c), and vase life (d) in 'Jumilia' and 'Samurai' cultivars. In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Percentage changes in fresh weight

Applying $\text{Ca}(\text{NO}_3)_2$ via foliar spray boosted the initial fresh weight of the flowers and slowed down the subsequent decline in relative fresh weight (Fig. 5). The greatest rise in fresh weight was observed after six days, after which the initial fresh weight began to decrease. This reduction was more pronounced in the control plants of both the cultivars (Fig. 5). The treatment that showed the highest effectiveness in increasing the initial fresh weight after 6 days in 'Samurai' was the application of 160 mg/L $\text{Ca}(\text{NO}_3)_2$, leading to an 11% rise in fresh weight. In contrast, for the 'Jumilia' cultivar, the most effective treatment was 80 mg/L $\text{Ca}(\text{NO}_3)_2$, leading to a 14.4% increase in the initial fresh weight (Fig. 5). After 10 days, the percentage of fresh weight reduction in untreated flowers was higher than that of the others, reaching less than the measured weight at the time of harvest (Fig. 5).

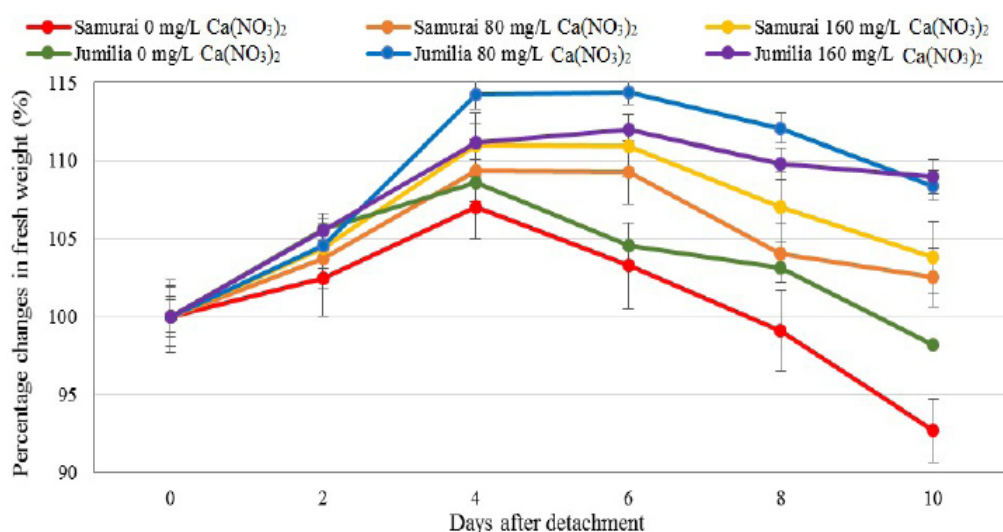


Fig. 5. Effects of foliar spray with concentrations of 0, 80, and 160 mg/L $\text{Ca}(\text{NO}_3)_2$ on percentage changes in fresh weight in 'Jumilia' and 'Samurai' cultivars. In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

DISCUSSION

The marketability and commercial value of cut rose flowers are determined by key attributes such as flower diameter, stem length, and flower color. In this study, foliar application of calcium nitrate $\text{Ca}(\text{NO}_3)_2$ significantly enhanced these quality parameters. Calcium plays a critical role in improving leaf count, which expands the photosynthetically active area and supports higher rates of photosynthesis. The elevated photosynthetic rate can provide energy for the stimulated growth of plant (Haghighi *et al.*, 2023). Here, a higher rate of photosynthesis in plant supplied with foliar Ca was associated with increases in diameter and length of the stem, flower, rosehip, and pedicel, which, in turn, improved the quality of the produced roses. These findings align with Kumar and Haripriya (2010), who reported that photosynthetic substances are transported to various plant parts, enhancing plant height. Similarly, Youssef *et al.* (2017) observed that foliar application of calcium chloride significantly increased growth parameters in lettuce, including plant length and leaf count. Additionally, our findings are consistent with observations in *Eustoma grandiflorum*, where foliar calcium treatments improved leaf number, chlorophyll content, and overall plant performance (Seydmohammadi *et al.*, 2020).

Foliar application of $\text{Ca}(\text{NO}_3)_2$ enhanced leaf calcium concentration, which is directly linked to increased photosynthetic activity in treated plants. Calcium regulates stomatal function, facilitating efficient gas exchange and influencing enzymes such as sedoheptulose-1,7-bisphosphatase and fructose-1,6-bisphosphatase in the Calvin cycle (Kreimer *et al.*, 1988; Wang *et al.*, 2019). In this regard, Ca spraying in apple resulted in a 57.4% increase in photosynthesis and a 36.8% increase in transpiration (Wang *et al.*, 2022). Improvements in transpiration rate (33%) and photosynthesis rate (28.7 %) in Ca-treated wheat have also been reported by Dolatabadian *et al.* (2013). These physiological improvements underscore the role of calcium in promoting carbon assimilation and plant vigor.

Foliar application of $\text{Ca}(\text{NO}_3)_2$ significantly increased Ca uptake by leaves and petals of rose flowers, and Ca accumulation was positively correlated with increased Ca concentration (Table 5). Foliar spraying allows plants to rapidly absorb nutrients through leaf tissues, as demonstrated in lettuce treated with calcium chloride, which exhibited a notable increase in leaf Ca levels (Fageria *et al.*, 2009; Youssef *et al.*, 2017). Additionally, another investigation has documented the effect of Ca source on increasing Ca levels in the leaves and petals of rose flowers (Banijamali *et al.*, 2018). This effect is attributed to enhanced transpiration, which facilitated Ca transport to petals, albeit at lower concentrations than in leaves. This unequal distribution is largely due to the higher transpiration rate in leaves, driven by their larger surface area, which makes them more effective at diverting Ca from flowers (Mc Laughlin and Wimmer, 1999). This leads to a several-fold increase in the concentration of Ca in the leaves than in the petals.

Despite the lower Ca concentration in petals, the levels were sufficient to enhance anthocyanin synthesis, particularly in 'Jumilia'. This cultivar-specific response highlights the role of genetic differences in regulating anthocyanin production under Ca treatments. Notably, at all levels of $\text{Ca}(\text{NO}_3)_2$ treatment, the anthocyanin content in 'Samurai' was higher than in 'Jumilia', attributable to genetic differences between the two varieties (Fig. 2c). Fertilization during the pre-harvest period serves as a key determinant of anthocyanin accumulation by regulating the expression of genes responsible for their production (Łysiak, 2022). Previous studies, such as Akbari *et al.* (2013), have shown that Ca plays a pivotal role in enhancing anthocyanin content in gerbera petals. In other words, raising the Ca concentration in cells stimulates the anthocyanin production. This aligns with our study's findings, which showed higher Ca levels in petals correlating with increased anthocyanin levels.

The foliar application of $\text{Ca}(\text{NO}_3)_2$ also improved leaf RWC, a critical indicator of plant water status (Fig. 3a). Calcium contributes to osmotic regulation, accounting for up to 19% of total osmotic adjustment (Wu *et al.*, 2023). Our findings align with previous studies where Ca treatment improved RWC in lettuce (Youssef *et al.*, 2017) and gerbera petals, likely by reducing stem blockage and enhancing lignin biosynthesis (Aghdam *et al.*, 2019). In the present study, the improved RWC in roses indicates enhanced water absorption and retention, which is essential for maintaining tissue hydration and delaying senescence.

An increased concentration of $\text{Ca}(\text{NO}_3)_2$ resulted in a significant upward trend in MSI, indicating delayed senescence in roses treated with calcium. Calcium stabilizes cell membranes and walls by integrating into their structures and reducing lipid peroxidation (Sairam *et al.*, 2011). Our findings align with those of Torre *et al.* (1999), who noted that calcium treatment delayed petal wilting by maintaining membrane integrity. Additionally, pre-harvest calcium application has been shown to reduce cell membrane damage and extend vase life in roses (Abdolmaleki *et al.*, 2015; Khosravi *et al.*, 2024).

Oxidative stress, caused by an imbalance between ROS generation and antioxidant defense mechanisms, leads to cellular damage and degradation (Ahmad *et al.*, 2019). Our study demonstrated that the highest activities of POD, SOD, and CAT enzymes were observed in the 'Samurai' cultivar following foliar application of 160 mg/L $\text{Ca}(\text{NO}_3)_2$ (Fig. 4a, b, and c). This treatment also resulted in the most significant percentage increase in vase life (Fig. 4d). This aligns with findings in gladiolus, where calcium treatments reduced ROS levels, enhanced membrane stability, and extended postharvest longevity (Sairam *et al.*, 2011). The role of preharvest calcium in improving postharvest shelf life has also been reported in other plants such as blueberries, tomato, and *Agaricus bisporus* (Mazumder *et al.*, 2021; Lobos *et al.*, 2021; Duan *et al.*, 2024). Importantly, the observed improvement in antioxidant activity underscores calcium's pivotal role in mitigating oxidative stress, enhancing membrane stability, and delaying the downstream effects of flower senescence. The observed effects can be attributed to foliar-applied $\text{Ca}(\text{NO}_3)_2$, as its absorption directly influences leaf and petal physiology, bypassing the root system.

The decline in flower weight, primarily attributed to reduced water absorption during senescence, was effectively alleviated by $\text{Ca}(\text{NO}_3)_2$ treatment, especially at higher concentrations. By improving water flow, calcium increased the initial fresh weight of rose flowers and delayed subsequent weight reduction (Fig. 5). This supports findings by Torre *et al.*, (1999), who reported that calcium treatment improved relative fresh weight in roses. The extended vase life of the rose cultivars in this study (42.8% for 'Jumilia' and 46.7% for 'Samurai') underscores calcium's multifaceted role in enhancing cell wall stability and reducing oxidative stress through increased antioxidant activity. Comparable benefits have been observed in other cut flowers, such as a 34% increase in rose vase life (Torre *et al.*, 1999) and a 21% increase in lilies, indicating the key role of calcium in improving vase life (Zhang *et al.*, 2018).

CONCLUSION

The findings indicated that foliar spraying with $\text{Ca}(\text{NO}_3)_2$ enhanced transpiration, leading to increased Ca uptake by rose petals and leaves. Spraying 160 mg/L $\text{Ca}(\text{NO}_3)_2$ solution increased photosynthesis, which helped improve growth parameters of 'Jumilia' and 'Samurai'. Additionally, the use of $\text{Ca}(\text{NO}_3)_2$ resulted in an increase in anthocyanin concentration in 'Jumilia'. $\text{Ca}(\text{NO}_3)_2$ plays an effective role in delaying the reduction in fresh weight of flowers by increasing RWC. Foliar spray with $\text{Ca}(\text{NO}_3)_2$, particularly at a concentration of 160 mg/L, enhances antioxidant defense mechanisms, leading to an enhancement of the membrane stability

index and consequently improves the vase life of roses. Overall, pre-harvest foliar spraying with $\text{Ca}(\text{NO}_3)_2$, by increasing photosynthetic activity, improves the quality of produced roses and delays the senescence of cut roses after harvest by activating antioxidant enzymes and enhancing membrane stability.

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