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Effects of foliar and fertigation based calcium nitrate applications on growth, flower quality, and postharvest physiology of poses in a soilless system

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ABSTRACT

This study investigated the effects of foliar and fertigation-based calcium nitrate applications on growth, flower quality, and postharvest physiology of two Rosa hybrida cultivars (‘Samurai’ and ‘Jumilia’) grown in a soilless system. Treatments included a control (10% of the recommended Ca level), foliar spraying, and fertigation, arranged in a factorial design with four replications. The experiment was conducted in a greenhouse at the Faculty of Agriculture, Ferdowsi University, Mashhad, Iran. Results revealed that root morphological characteristics such as length and volume were maximized in the ‘Jumilia’ cultivar when calcium nitrate was supplied via fertigation. Conversely, foliar spraying with calcium nitrate markedly improved shoot and floral characteristics. Compared to the control, foliar calcium nitrate increased flower diameter by 15.4%, enhanced hip length by 14.25 cm, and increased hip diameter in the ‘Samurai’ cultivar by 18.76%. Moreover, foliar application of calcium nitrate extended the vase life of cut roses to about 10 days by preserving higher relative fresh weight. Antioxidant enzyme activities were also enhanced under foliar treatment in the ‘Samurai’ cultivar, with superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) increasing by 53.84%, 75.71%, and 97.28%, respectively. Additionally, foliar-sprayed ‘Samurai’ plants showed nearly double calcium accumulation in leaves and petals. Interestingly, in the ‘Jumilia’ cultivar, foliar application reduced leaf zinc content by 68.87%, while simultaneously enhancing leaf magnesium concentration by 64.16%. Overall, foliar supplementation with calcium nitrate proved to be a practical strategy for improving flower quality, enhancing antioxidant defense, and prolonging vase life of roses in soilless culture.

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1. Introduction

Roses (Rosa hybrida) are members of the Rosaceae family and represent a highly diverse group, consisting of nearly 200 species and more than 18,000 recognized cultivars across the globe. Currently, the cultivation and trade of roses ranks first in most countries of the world (Farazmandi et al., 2020). Roses recognized as the garden ornaments due to the variety of different characteristics including flower form, dye, odour and scent. In additions, they are extremely used in perfume and beautifying industries. Rose cultivation goes back millions of years and as a major cut flower crop, it acclimates to various climatic conditions (Desta et al., 2022; Ghadimian and Danaei, 2020; Hong et al., 2021). Recent studies show that nutrition, light spectrum, and postharvest treatments influence cut rose quality and vase life. A red-dominant LED (90:10 red-to-blue) improved photosynthesis, pigments, and carbohydrates, extending vase life by up to 30% (Davarzani et al., 2025). Melatonin pulse treatments reduced ion leakage and maintained water status and pigments (Shamsinejad et al., 2024), while gaseous enrichment of vase solutions with ethylene inhibitors and bioactive compounds mitigated oxidative stress and prolonged vase life (AsgariI Gouraj, 2025), further highlighting the benefit of integrating nutritional, environmental, and postharvest management strategies for improved cut flower performance.

Cut roses are highly perishable and have a limited vase life. In commercial rose production, achieving desirable growth characteristics—such as adequate stem length, appropriate stem diameter, and optimal flower diameter—is essential for meeting market standards and ensuring high-quality cut stems. Therefore, alongside postharvest quality, there is an urgent need to enhance both vegetative and floral attributes as well as to extend the vase longevity of rose flowers in order to supply satisfactory products to the marketplace. Numerous studies have addressed these goals and explored various approaches to improve rose quality and longevity (Ehsanimehr et al., 2024; Ha et al., 2024).

Calcium as an essential macronutrient element plays the important roles in cell membrane penetrability, protein biosynthesis, enzyme activities, regulating ion transduction, use of photo assimilates and energy transition (Ahmad & Rab, 2020; Khalaj et al., 2023; Mahajan& Pal, 2020). Moreover, reinforced cell walls and increased thickness are the other roles of calcium in plants (Mahajan & Pal, 2020). It also affects cell wall integrity and is regarded as the primary barrier to cell breakdown (Abdolmaleki et al., 2015). Therefore, it is imperative to recognise the most suitable rose cultivars for profitable cultivation in landscape, and it is also crucial to determine the appropriate calcium dosage for rose plants to enhance yields and overall performance

Recent studies confirm that calcium plays a central role in maintaining cell wall structure and improving water relations in cut flowers. For instance, Ehsanimehr et al. (2024) demonstrated that pre-harvest foliar application of calcium significantly enhanced cell wall integrity and reduced fresh weight loss in cut roses, thereby extending vase life. Moreover, Ha et al. (2024) found that calcium accumulation in petal and leaf tissues is closely associated with delayed senescence and improved structural stability in cut roses under specific light regimes. These recent findings collectively highlight that calcium deficiency weakens cell wall structure, reduces water uptake, and ultimately shortens the postharvest life of cut flowers. Calcium deficiency can lead to disorders in terminal plant sections and growing branch tips, resulting in fruit softness, stem bending, and reduced postharvest longevity in fruits and cut flowers. Recent hydroponic studies have shown that nutrient management, including the choice of nitrogen sources and careful pH adjustment, can influence calcium uptake and overall plant performance (Soufi et al., 2025).

Foliar feeding ensures rapid nutrient absorption and minimizes nutrient antagonism. Recent studies show that foliar micronutrients significantly improve plant physiology—for example, Fe and Zn oxides enhanced root growth and antioxidant activity in sorghum (Dehestani et al., 2025), while humic acid with zinc sulphate increased nutrient uptake and chlorophyll in Physalis (Kazemi et al., 2024). These findings highlight foliar nutrition as an efficient strategy to improve plant performance. In addition, foliar spraying of mineral elements avoids the pollution of groundwater through decreasing the nutrient leaching and subsequently decreases fertilizer expense (Mahajan & Pal, 2020). In contrast, fertigation provides simultaneously water and essential nutrients required for plant growth and development. The precise incorporation of water and nutrients is important for great yield and quality. Fertigation improves nutrient use efficiency, reduces fertilizer losses, and enhances nutrient uptake and growth in greenhouse crops (Ramasubramanian et al., 2025). Since calcium is an immobile element in plants, the foliar spraying can be useful to enhance its absorption. Previous studies reported the impact of pre-harvest foliar application of calcium on rose floral characteristics (Banijamali et al., 2018). The increased calcium absorption by leaves and stem results from spraying calcium nitrate and calcium chloride which had a direct impact on the growth attributes, vase life of cut flowers and method of application (Khalaj et al., 2023). Stem diameter and stem firmness in Gerbera jamesonii Bolus were increased using calcium treatment (Combrink, 2018; Khalaj & Noroozisharaf, 2020). Based on available references, exogenous application of calcium participates in water absorption by cut stems through stem lignification (Roosta et al., 2024). Additionally, external application of calcium delayed stem bending in gerbera cut flowers (Gerbera jamesonii cv. Tamara), likely due to the cross-linking of pectin molecules and subsequent cell wall strengthening (Khalaj et al., 2017). On the other hand, calcium nitrate supplies available

nitrogen, which enhances energy production (Watane et al., 2022). Application of calcium nitrate extended the postharvest life and raised bud opening in cut rose flower (Robichaux, 2008).

Although calcium plays multiple essential roles in plants, it its content is low in petal and if its content increases in the aerial organs, the postharvest life and the quality attributes of the flowers will be improved. Due to calcium's low mobility, calcium fertigation alone is often inefficient, making foliar spraying a more effective approach.. So, spraying method is better and should be considered. In most of the studies, calcium is applied as a spray and supplement, and it is not removed from the roots as well. However, in this study, we completely removed it from the roots and just applied a small amount of calcium to meet the needs of the roots. To our knowledge, no previous studies have examined the effects of different calcium nitrate application methods on floral quality, antioxidant enzyme activity, and vase life. This study is the first to evaluate these parameters in Rosa hybrida under hydroponic conditions using both fertigation and foliar spraying of calcium nitrate. The main objective of this study is to determine whether foliar calcium application can compensate for reduced calcium levels in the nutrient solution and whether this approach can enhance flower quality and postharvest longevity.

2. Materials and Methods

The study was conducted using two grafted rose cultivars (Rosa hybrida L.), "Samurai" and "Jumilia," grown in a hydroponic system with 100% perlite (particle size 3–5 mm). Planting commenced on February 14th, and the experiment continued for one year to observe plant growth and development. The physical and chemical properties of the perlite medium employed are detailed in Table 1. To ensure proper establishment and acclimatization of the plants, both cultivars were irrigated with tap water for the first three days following planting. This initial irrigation aimed to minimize transplanting stress and promote uniform root development.

Table 1. Pphysical and chemical properties of the growth medium utilized in this study.

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

Initially, the plants were treated with an N:P:K fertilizer at a 10:50:10 ratio to enhance root development and were protected against fungal infections by applying methyl thiophanate fungicide (0.5 ml/L) within the first week. To ensure uniform establishment, the rose cultivars were fertigated with a modified half-strength Hoagland solution for 30 days. After this phase, all plants were bent above the third bud to standardize growth. From that point until the end of the experiment, plants received full-strength Hoagland solution, except in the control and foliar spray treatments, where calcium nitrate was limited to 10% of the total fertilizer requirement (Table 2). Calcium nitrate was applied according to the treatment plan, and potassium nitrate was used to balance nitrogen levels when calcium was reduced.

Table 2. The nutritional regimen employed for plant cultivation was formulated based on the Hoagland solution, ensuring a balanced supply of essential macro- and micronutrients.

compound	Concentration of stock Solution (gL^{-1})	Volume of Stock Solution Per Liter of Final Solution (ml)	Element	Final concentration of Element Macro($mmolL^{-1}$) Micro($\mu molL^{-1}$)
			N	16
KNO3	101.10	6	K	6
Ca(No3)2-4H2O	236.16	4	Ca	4
NH4H2Po4	115.08	2	P	2
MgSo4-7H2O	246.49	1	S	1
			Mg	1
KCl	1.864	2	Cl	50
H3Bo3	0.773	2	B	25
MnSo4-H2O	0.169	2	Mn	2
ZnSo4-7H2O	0.288	2	Zn	2
CuSo4-5H2O	0.062	2	Cu	0.5
H2MoO4(85%MoO3)	0.040	2	Mo	0.5
NaFeDTPA(10%Fe)	30.0	0.3-1	Fe	18-54

The pH and electrical conductivity (EC) of the nutrient solution were maintained at 5.8–6.0 and 1.5–2.0 mS cm^{-1}, respectively, and were monitored daily throughout the experiment. Adjustments were made as needed to maintain consistent conditions. The greenhouse was fitted with climate control systems, maintaining daytime and nighttime temperatures at 25 ± 2 °C and 16 ± 2 °C, respectively. Relative humidity was kept at 65% to 70%, and the average photosynthetic photon flux density (PPFD) of natural light within the greenhouse was 240 ± 5 µmol m^{-2} s^{-1}.

The experiment was conducted as a factorial arrangement within a completely randomized design (CRD) with two factors:

Factor A: Rose cultivar ('Samurai' and 'Jumilia')

Factor B: Calcium nitrate application method with three levels:

1. Control (10% of the calcium requirement in the nutrient solution)
2. Foliar spray (10% of calcium in the nutrient solution + foliar application)
3. Fertigation (100% of calcium in the nutrient solution)

Each treatment was replicated four times, and each replication included a fixed number of plants per treatment. In the control and foliar treatments, only 10% of the calcium requirement was supplied via the nutrient solution, and potassium nitrate was used to compensate for the nitrogen removed when calcium levels were reduced. Foliar applications of calcium nitrate were performed weekly between 7:00 and 8:00 a.m. according to the Hoagland solution recommendations (Table 3).

Table 3. Experimental Treatments:

Treatment	Cultivar	Ca Application Method	Ca Level in Nutrient Solution	Notes on N Compensation
T1	Samurai	Control	10%	N balanced with KNO_3
T2	Samurai	Foliar spray	10%	N balanced with KNO_3
T3	Samurai	Fertigation	100%	Full N in solution
T4	Jumilia	Control	10%	N balanced with KNO_3
T5	Jumilia	Foliar spray	10%	N balanced with KNO_3
T6	Jumilia	Fertigation	100%	Full N in solution

Figure 1. Rose plants under different calcium nitrate treatments in the greenhouse

2.1. Root volume and length

Root length was determined using a standard ruler and reported in centimeters. For root volume, the entire washed root system of each plant was carefully placed in a graduated cylinder containing a known volume of water, and the increase in water level was recorded as the total root volume. This approach offers a simple yet accurate estimation of root growth and development.

2.2. Assessment of Floral Traits

All harvested flowers per treatment and replication were measured. Flowers were collected at full bloom, specifically when the sepals had curved downward and the blooms had assumed a cylindrical form. Various floral characteristics were evaluated, including the length and diameter of rose hips, stem length and diameter, as well as flower length and diameter, alongside vase life. Stem length was measured using a standard ruler, whereas a digital caliper was employed to determine flower, stem, and hip diameters and lengths to ensure precise and accurate measurements. These observations allowed for a comprehensive assessment of flower morphology and overall quality.

2.3. Flower vase life

Cut flowers were placed in plastic bottles containing 500 mL of distilled water and maintained under controlled conditions, with a temperature of 25 ± 2 ℃, relative humidity of 65 ± 5%, and a 12-hour photoperiod provided by fluorescent lamps at 20 µmol m^{-2} s^{-1}. Vase life was evaluated by recording the number of days from harvest until senescence symptoms, such as bent neck or petal drop, became evident, allowing precise assessment of postharvest longevity and freshness retention in the rose cultivars.

2.4. Fresh weight

The fresh weight of rose flowers was measured every other day throughout the vase life period, with initial weights recorded immediately after harvesting and before placing the flowers into the solutions. Subsequent measurements were performed every other day up to the 10th day. The fresh weight of detached flowers was expressed relative to their initial weight to calculate the percentage of weight loss, providing an indicator of water retention and overall postharvest quality.

2.5. Greening index

Greening index was measured on day 60, 75 and 90 after calcium nitrate application by non-destructive method using chlorophyll estimation instruments SPAD (Soil Plant Analysis Development). Readings were taken from 10 randomly selected leaves per plant, representing different parts of the shoot, and values were averaged over the leaves and plants. New leaves were sampled at each time point; the same leaves were not measured repeatedly.

2.6. Mineral elements

Plant materials were initially oven-dried at 72 ℃ for 48 hours, then ground and dry-ashed at 500 ℃ for 4 hours. A 0.5 g portion of the ash was digested in 10 mL of 2N hydrochloric acid (HCl) and diluted to 50 mL with distilled water. The concentrations of calcium (Ca), iron (Fe), manganese (Mn), and zinc (Zn) were subsequently determined using inductively coupled plasma mass spectrometry (ICP-MS) (Volpin & Elad, 1991).

2.7. Anthocyanin content

Anthocyanin content in rose petals was determined following the method described by Wagner (1979). In the post-harvest period, 1000 mg of fresh petals were treated with 10 mL of methanol and kept in the dark at 4 ℃ overnight to allow thorough extraction. The mixture was then centrifuged at 4000 rpm for 10 minutes to remove solid particles. Quantification of anthocyanins was carried out using a spectrophotometer at a wavelength of 550 nm, providing an accurate measure of pigment concentration in the petal tissue.

2.8. Antioxidant enzymes activity

In the post-harvest period, frozen petal samples (0.2 g) were homogenized in 5 mL of 50 mM sodium phosphate buffer (pH 7.8) to obtain a uniform extract, which was subsequently centrifuged at 13000 × g for 20 minutes at 4 ℃. The supernatant obtained was used to determine the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). SOD activity was measured using a 3 mL reaction mixture containing 50 mM phosphate buffer (pH 7.8), 1.3 µM riboflavin, 63 µM nitro blue tetrazolium chloride (NBT), 13 µM methionine, and the enzyme extract. The mixture was illuminated, and absorbance at 560 nm was recorded. SOD activity was expressed as Units per mg protein, where one Unit is defined as the amount of enzyme causing 50% inhibition of NBT photoreduction per mg protein. CAT activity was determined with a mixture of 50 µL enzyme extract, 50 mM potassium phosphate buffer (pH 7), and 15 mM hydrogen peroxide. The reaction commenced upon adding the enzyme extract, and the decomposition of hydrogen peroxide was monitored at 240 nm. Results were reported as µmol min^{-1} mg^{-1} protein using an extinction coefficient of 39.4 mM^{-1} cm^{-1}. POD activity was assayed according to Chen et al. (2009) with a 3 mL mixture containing 0.2% o-dianisidine, 0.1 mM potassium phosphate buffer (pH 7.4), and the enzyme extract. Absorbance changes at 470 nm were measured over 1 min, and activity was expressed as µmol min^{-1} mg^{-1} protein. This approach ensures that enzyme activities (SOD, CAT, POD) are clearly quantified with defined units, consistent with values presented in figures and tables.

2.9. Statistical analysis

All experimental data were analyzed using SAS software version 9.4. Differences among treatment means were compared using the Least Significant Difference (LSD) test at a 5% significance level ($p \leq 0.05$) when the ANOVA F-test was significant. This approach enabled identification of statistically significant variations between treatments and ensured a rigorous evaluation of the results.

3. Results

3.1. Root volume and length

Based on the ANOVA results, the interaction between the calcium nitrate application method and rose cultivar significantly influenced root volume. The effects of various calcium nitrate application methods on root length are summarized in Table 4. Among the treatments, the "Jumilia" cultivar fertigated with calcium nitrate showed the highest root volume (102.37 m³), significantly exceeding all other treatments. This suggests that fertigation promotes root development. Conversely, the lowest root volume was recorded in the "Samurai" cultivar subjected to foliar calcium nitrate application (Figure 2a). In terms of root length, plants treated with foliar calcium nitrate had the shortest roots (27.95 cm), whereas those fertigated with calcium nitrate (34.26 cm) or in the control group (31.62 cm) exhibited the longest roots (Table 4). These results indicate that fertigation with calcium nitrate is more effective in enhancing root growth, particularly in the "Jumilia" cultivar.

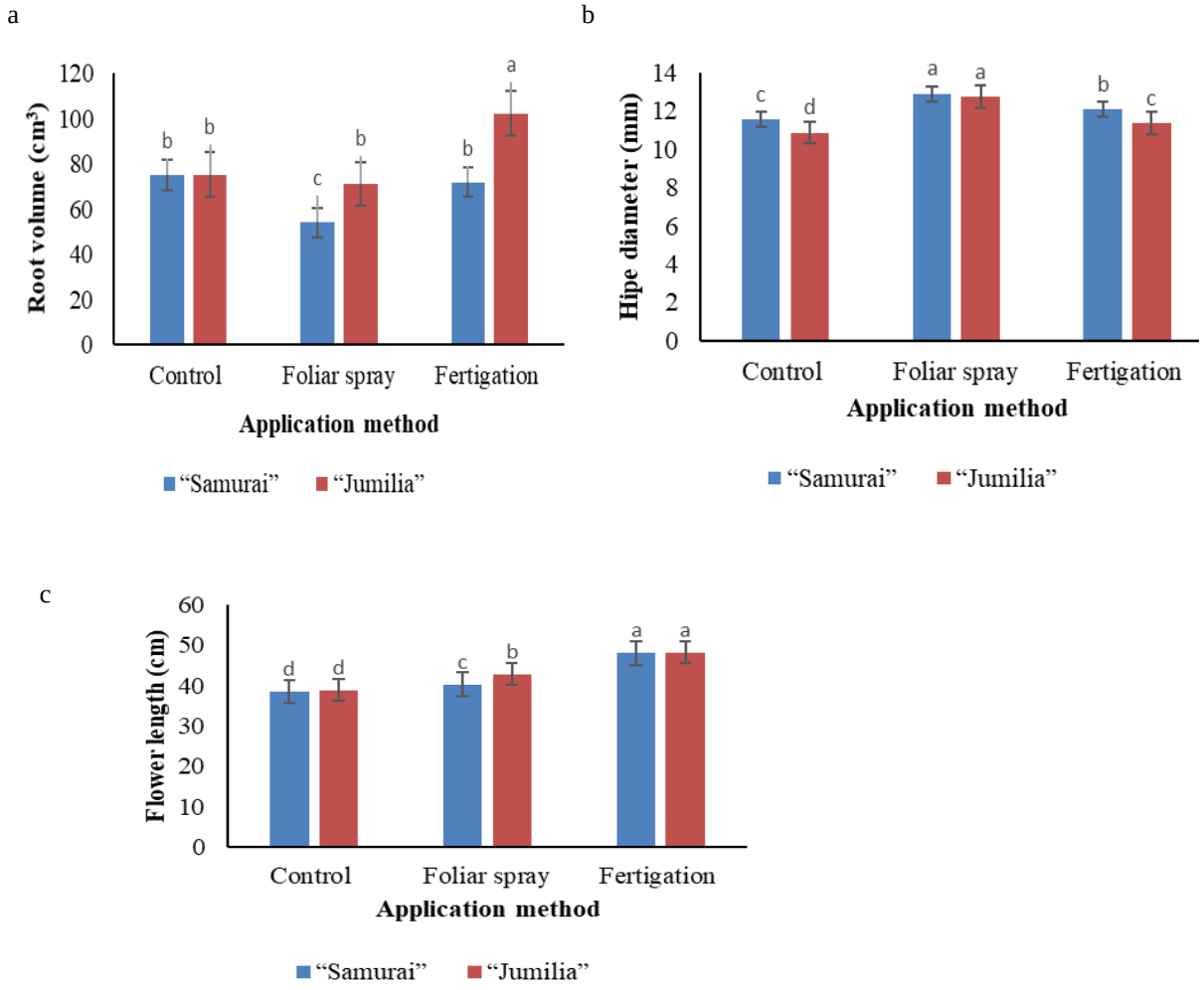


Figure 2. Impact of application method of calcium nitrate and cultivar on flower length (cm), hip diameter (mm), root volume (cm³) in rose flower

Table 4. Impact of application method of calcium nitrate on root length (cm) and flower diameter (mm) of rose flower

Application method	Root length (cm)	Flower diameter(mm)
Control	31.62± 2.32A	30.97±0.68B
Foliar spray	27.95± 1.83B	35.74± 1.93A
Fertigation	34.26±2.97A	35.21±3.59A

Within each column, values marked with the same letter are not significantly different based on the LSD test at $P < 0.05$.

3.2. Rose hip length and diameter

Statistical analysis revealed that both the calcium nitrate application method and rose cultivar had a significant impact on hip length. Foliar spraying of calcium nitrate markedly enhanced hip length (14.25 cm) compared to other application approaches. Furthermore, the "Samurai" cultivar displayed longer hip length than the "Jumilia" cultivar (Figure 3).

Hip diameter was significantly affected by the interaction between calcium nitrate application method and cultivar. In the "Samurai" cultivar, foliar spraying of calcium nitrate resulted in an 18.76% increase in hip diameter compared to the lowest measurement of 10.87 mm observed in the control "Jumilia" plants (Figure 2b). Conversely, foliar application did not significantly affect hip diameter in the "Jumilia" cultivar, indicating that the effect of calcium nitrate on hip size varies between cultivars, with "Samurai" responding more strongly to foliar treatment.

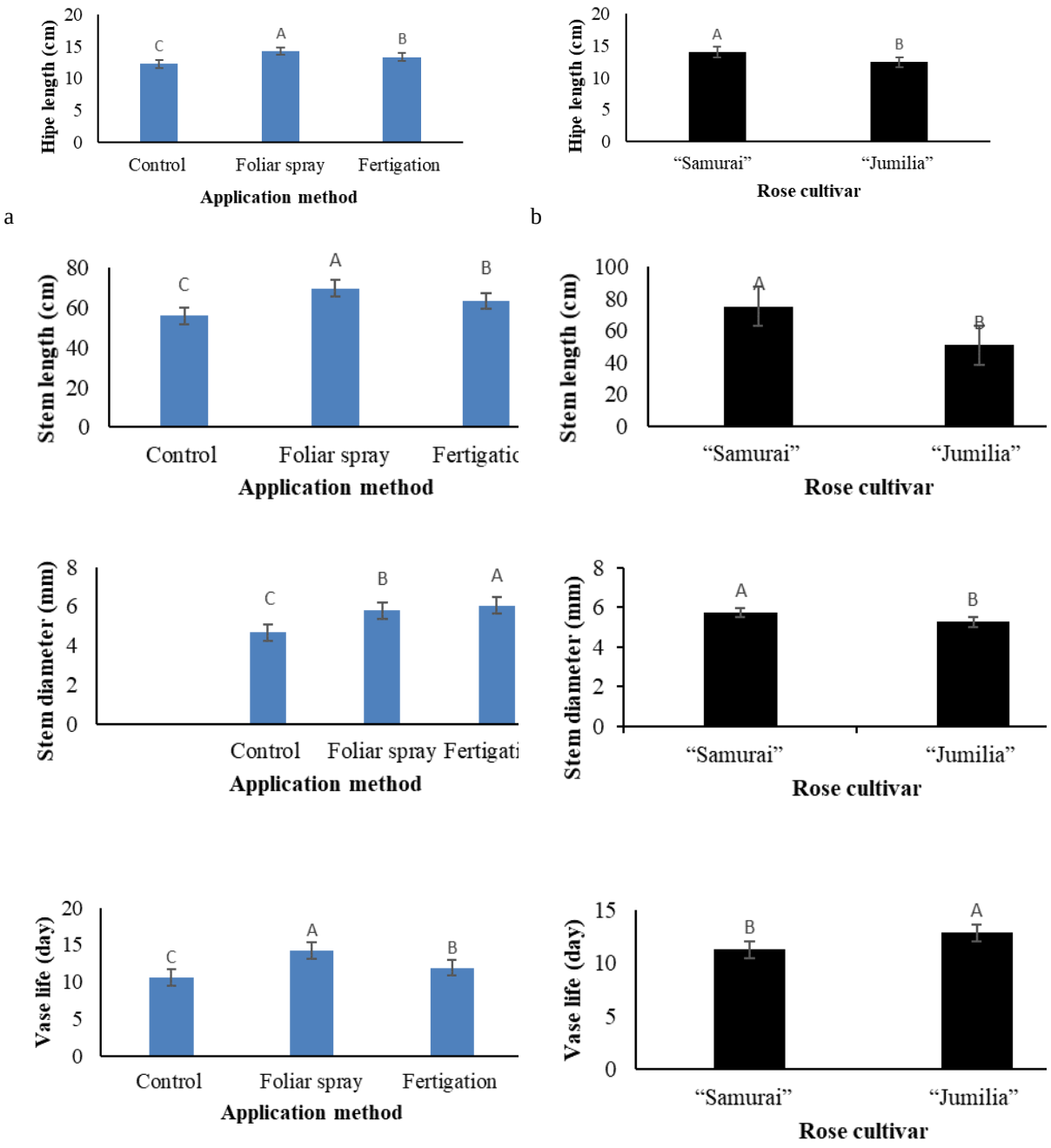


Figure 3. Effect of application method of calcium nitrate (a) and cultivar (b) on stem length (cm), stem diameter (mm), hip length (cm) and vase life (day) in rose flower

3.3. Stem length and diameter

Stem length and diameter of rose flowers were significantly affected ($P < 0.05$) by both the cultivar and the method of calcium nitrate application (Figure 3). However, the interaction between cultivar and application method was not significant for these traits (data not shown). Among the cultivars, "Samurai" exhibited greater stem length and diameter, measuring 75.27 cm and 5.72 mm, respectively, compared to "Jumilia". Among the different treatments, plants receiving calcium nitrate showed the greatest stem length (69.79 cm), whereas the control treatment resulted in the shortest stems (55.89 cm) (Figure 3). Interestingly, the fertigation method led to a 29.61%

increase in stem diameter compared with other application methods, highlighting the effectiveness of fertigation in promoting stem thickening.

3.4. Flower length and diameter

The “Jumilia” cultivar fertigated with calcium nitrate exhibited a 24.94% increase in flower length. However, this value was not significantly different from that of the “Samurai” cultivar under fertigation. In both cultivars, the control treatment produced the shortest flowers (Figure 2c).

ANOVA results indicated that the method of calcium nitrate application had a significant effect on flower diameter. Nonetheless, no significant differences were observed between cultivars or in the interaction between application method and cultivar (data not shown). Plants receiving foliar calcium nitrate exhibited the largest flower diameter (35.74 mm), which was not significantly different from the diameter observed in the fertigation treatment (Table 4). These findings suggest that both foliar and fertigation applications of calcium nitrate can enhance flower size, while the cultivar type has a lesser impact under these experimental conditions.

3.5. Relative fresh weight

During the 10-day postharvest period, both ‘Samurai’ and ‘Jumilia’ cultivars treated with different calcium nitrate applications exhibited the lowest relative reduction in fresh weight. In contrast, flowers of these cultivars maintained in distilled water exhibited a greater loss in fresh weight (Figure 4). These results indicate that calcium nitrate application, regardless of the method, effectively helps maintain water content and prolongs the postharvest freshness of rose flowers.

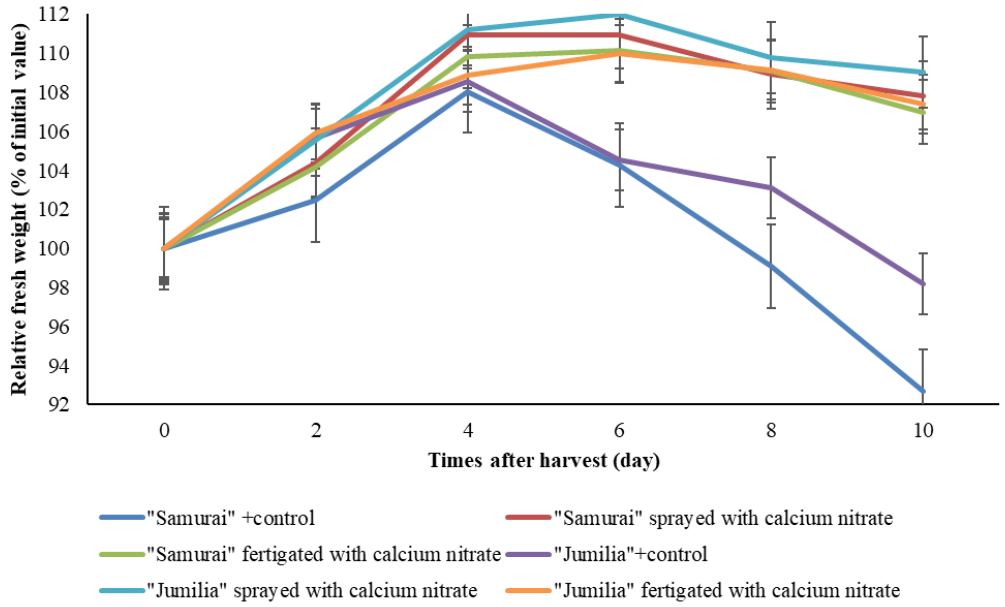


Figure 4. The relative fresh weight of two cultivar roses influenced by various application methods of calcium nitrate in the nutrient solution at different times after harvest.

3.6. Flower vase life

Vase life of the rose flowers was significantly affected ($P < 0.05$) by both the cultivar and the calcium nitrate application method (Figure 3). However, the interaction between application method and cultivar did not have a significant effect on vase life (data not shown). Foliar application of calcium nitrate increased flower vase life by 41.94% compared to the control treatment. Additionally, the “Jumilia” cultivar exhibited a longer vase life (12.85 days) than the “Samurai” cultivar (11.28 days) (Figure 3). These results indicate that foliar application is more effective than fertigation in enhancing postharvest performance, likely due to improved calcium availability in aerial tissues.

3.7. Greening index

Both the rose cultivar and the calcium nitrate application method significantly ($P < 0.05$) affected the leaf greening index. However, the interaction between application method and cultivar was not significant (data not shown). Application of calcium nitrate, whether via foliar spray or fertigation, resulted in higher leaf greening index values than the control on days 60, 75, and 90 (Table 5). Furthermore, the “Jumilia” cultivar consistently exhibited higher leaf greening index values on days 60, 75, and 90 (51.22, 53.12, and 54.99, respectively) than the

"Samurai" cultivar (Table 6). These results indicate that calcium nitrate application, irrespective of the method, enhances leaf chlorophyll retention, with "Jumilia" showing a stronger response.

Table 5. Effect of cultivar on greening index of rose flower

Cultivar	Greening index (60d)	Greening index (75d)	Greening index (90d)
"Samurai"	49.56±3.33B	51.44±3.60B	53.32±3.80B
"Jumilia"	51.22±3.35A	53.12±3.64A	54.99±3.61A

Within each column, values marked with the same letter are not significantly different based on the LSD test at $P < 0.05$.

Table 6. Impact of application method of calcium nitrate on greening index of rose flower

Application method	Greening index (60d)	Greening index (75d)	Greening index (90d)
control treatment	46.27±1.48B	47.66±1.52B	49.46±1.54B
Foliar spray	52.97±1.74A	54.61±1.47A	56.46±1.22A
Fertigation	51.94±2.07A	54.56±2.26A	56.56±2.50A

Within each column, values marked with the same letter are not significantly different based on the LSD test at $P < 0.05$.

3.8. Anthocyanin content

Anthocyanin levels in rose flowers were significantly influenced by cultivar ($P < 0.05$). The combined effect of calcium nitrate application method and cultivar had no significant impact on anthocyanin content (data not shown). Among the cultivars, "Samurai" accumulated more anthocyanins, reaching 130.72%, in comparison with "Jumilia" (Figure 5). These findings suggest that flower coloration is predominantly controlled by genetic factors, with "Samurai" exhibiting greater pigment deposition.

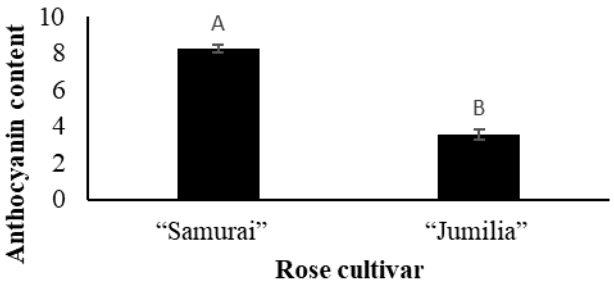


Figure 5. Impact of cultivar on anthocyanin content in rose flower

3.9. Antioxidant enzyme activity

In the "Samurai" cultivar, foliar spraying of calcium nitrate enhanced catalase (CAT) activity by 75.71% relative to the lowest activity observed in the control treatment of the same cultivar (1.40 µmol min^{-1} mg^{-1} protein) (Figure 5). Conversely, CAT activity in the "Jumilia" cultivar showed no significant differences between foliar spraying and fertigation treatments (Figure 6a). Peroxidase (POD) activity in the "Samurai" cultivar increased by 97.28% after foliar application of calcium nitrate, whereas the lowest POD activity (27.28 µmol min^{-1} mg^{-1} protein) was observed in the control treatment of the "Jumilia" cultivar (Figure 6b). ANOVA results indicated that both the method of calcium nitrate application and the cultivar significantly affected superoxide dismutase (SOD) activity. However, no significant variations were detected in the interaction between application method and cultivar (data not shown). SOD activity in the "Samurai" cultivar was 31.13% higher than in the "Jumilia" cultivar. Furthermore, foliar application of calcium nitrate increased SOD activity by 53.84% compared to the control treatment (Figure 6c, d). These findings demonstrate that foliar application of calcium nitrate effectively enhances the antioxidant defense system in rose petals, particularly in the "Samurai" cultivar, contributing to improved oxidative stress tolerance and potentially extending postharvest longevity.

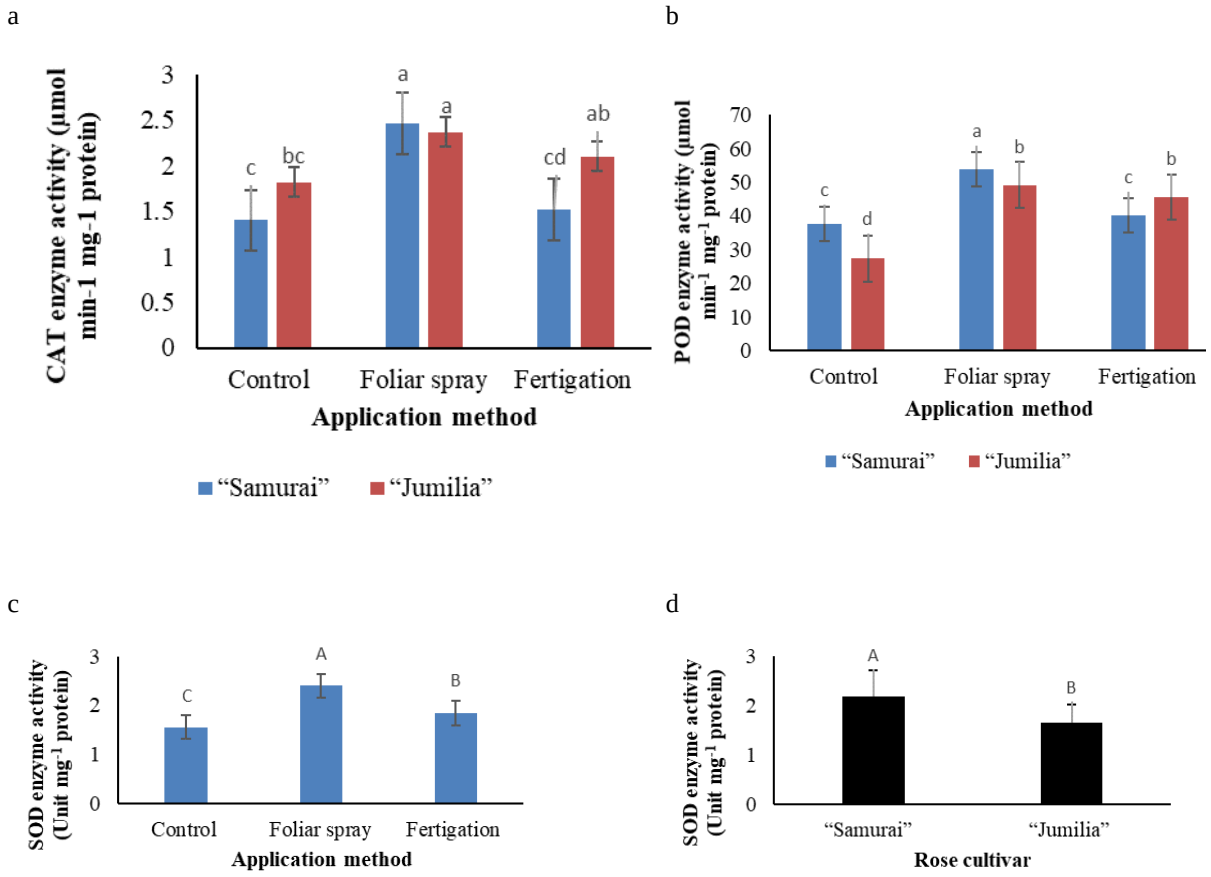
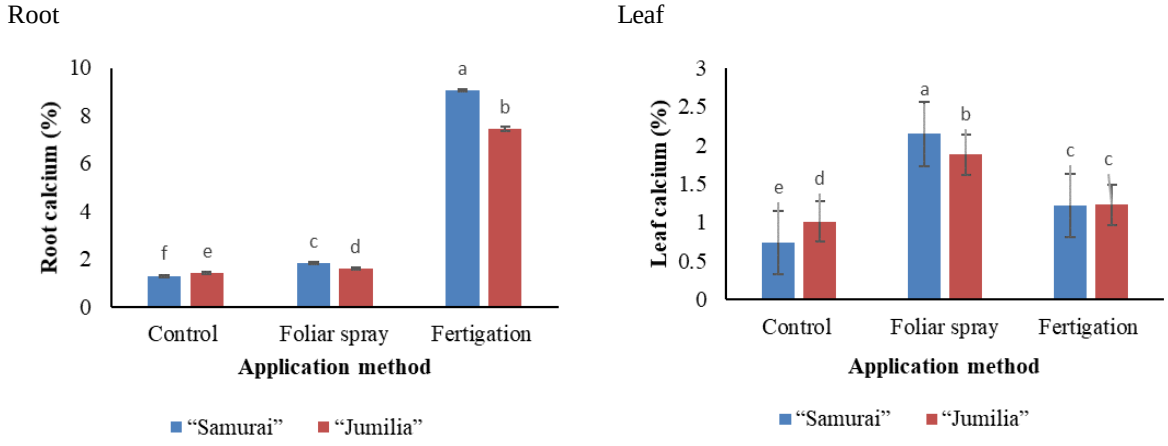


Figure 6. Effect of application method of calcium nitrate and cultivar on antioxidant enzymes activity in rose flower

3.10. Mineral elements

The calcium content in rose roots, leaves, and petals was significantly affected by the interaction between the cultivar and the method of calcium nitrate application. Specifically, foliar spraying of calcium nitrate in the "Samurai" cultivar led to an approximate twofold increase in calcium levels in both leaves and petals compared to the lowest values recorded in the control treatment (0.73% in leaves and 0.78% in petals) (Figure 7). Conversely, fertigation with calcium nitrate led to a remarkable 601.13% increase in root calcium concentration in the "Samurai" cultivar compared to the control (Figure 7). These results indicate that both foliar and fertigation applications of calcium nitrate effectively enhance calcium accumulation in different plant tissues, with the mode of application influencing the distribution of calcium within the plant.



Petal

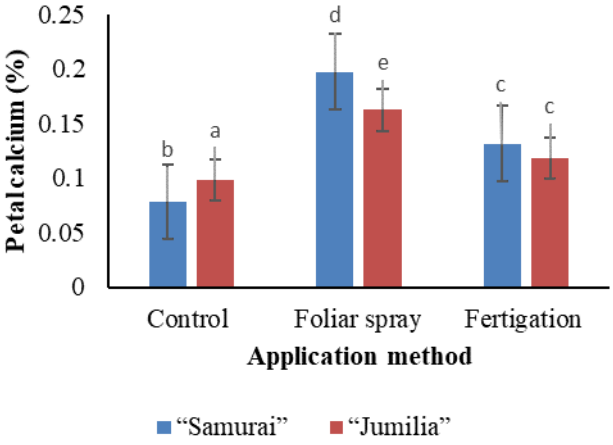


Figure 7. Effect of Calcium Nitrate Application Method and Cultivar on Calcium Content in Roots, Leaves, and Petals of Rose Flowers

In the "Jumilia" cultivar, applying calcium nitrate as a foliar spray led to a substantial reduction in leaf zinc content, reaching 68.87% of the maximum level recorded in the control treatment (Table 7). In contrast, foliar application of calcium nitrate in the "Samurai" cultivar caused a 32.30% increase in leaf iron content when compared to the "Jumilia" cultivar that received calcium nitrate via fertigation. Moreover, in the "Jumilia" cultivar, foliar spraying of calcium nitrate markedly enhanced leaf manganese levels by 64.16% relative to the lowest concentration observed in the control treatment (Table 7).

Concerning root mineral content, iron levels in the control treatment of the "Jumilia" cultivar were nearly three times higher than the lowest value (0.091%) observed in the same cultivar under calcium nitrate fertigation. Furthermore, foliar spraying of calcium nitrate in the "Samurai" cultivar led to a remarkable 470.51% increase in root manganese concentration compared to the control. Zinc levels in the roots showed variation among treatments, reaching a maximum of 0.115% in the "Samurai" cultivar subjected to foliar calcium nitrate application, while the minimum value of 0.008% was observed in the "Jumilia" cultivar under fertigation (Table 7).

These results indicate that calcium nitrate application method and cultivar significantly influence the distribution and accumulation of essential micronutrients in both leaves and roots, highlighting the interplay between fertilization strategy and genetic background.

Table 7. Effect of Calcium Nitrate Application Method and Cultivar on Iron, Manganese, and Zinc Content in Leaves and Roots of Rose Plants

		Leaf (%)			Root (%)		
Cultivar	Application method	Iron	manganese	zinc	Iron	manganese	zinc
"Samurai"	Control	0.028±0.0003b	0.011±0.0001d	0.018±0.0004b	0.172±0.004c	0.003±0.00008f	0.019±0.001c
	Foliar spray	0.030±0.0002a	0.011±0.0004d	0.015±0.0008c	0.242±0.003b	0.021±0.0001a	0.115±0.004a
	Fertigation	0.023.32±0.0002e	0.012±0.0003c	0.011±0.0017d	0.118±0.003d	0.005±0.0001e	0.014±0.002d
"Jumilia"	Control	0.027±0.0002c	0.008±0.0001e	0.024±0.0004a	0.253±0.003a	0.015±0.0002b	0.075±0.0002b
	Foliar spray	0.025±0.0002d	0.014±0.0003a	0.007±0.0002e	0.120±0.002d	0.008±0.00006d	0.017±0.001cd
	Fertigation	0.022±0.0002f	0.013±0.0003b	0.010±0.0009d	0.091±0.001e	0.009±0.00009c	0.008±0.001e

Within each column, values marked with the same letter are not significantly different based on the LSD test at $P < 0.05$.

4. Discussion

To obtain commercial roses with extended and powerful stems, their morphological characteristics during the cultivation should be considered. Thus, the loss of the marketable and commercial importance of the cut flowers followed from the loss of quality in plants' vegetative attributes (Sharifi and Naderi, 2019). Calcium as a divalent

cation, plays an important role in prevention of stem bending. Additionally, it adjusts the uptake of mineral elements across plasma cell membranes as well as participates in plant cell division and elongation, cell membrane composition and penetrability, nitrogen metabolism, and carbohydrate transduction (Khalaj et al., 2023).

The impact of spraying calcium nitrate on increased stem length of roses can be caused by an entrance of calcium into the structure of the middle plate in the cell wall, resulting in adhering adjacent cells that enhances the stem strength, in addition to advancing plant development (Al-Ibraheemi et al., 2021). Foliar spraying of calcium nitrate has been shown to enhance stem elongation in Rose Moss Portulaca Grandiflora, likely due to the effect of calcium ions in promoting both cell division and cell elongation (Al-Ibraheemi et al., 2021), which was in concurrent with the results from this research. Prior studies have shown that application of calcium nitrate effectively led to an increase in stem diameter followed from an intensification of the amount of thickened cell layers and the sclerenchyma cell walls (Zhao et al., 2019). The rise in the volume and length of the roots due to the calcium consumption can be attributed to the fact that the calcium enhances its level in the proximity of the roots to obtain an adequate calcium for the cell division process, which in turn increases the root growth and liveliness and consequently increases its dimensions (Johnson et al., 2019). Also, another reason for the improvement of most growth parameters through calcium nitrate application is the nitrogen that is involved in the structure of auxin, which results in the enhancement of cellular division and elongation, and which is also an important component of chlorophyll that affects photosynthesis, protein synthesis, nucleic acid production, and coenzyme formation (Mohammed & Abood, 2020). Therefore, it increases vegetative and reproductive growth (Azeez et al., 2017; Milioni et al., 2019; Saikia et al., 2018). Given to the role of calcium in plant cell elongation (Khalaj & Noroozisharaf, 2020; Marschner, 2012; Seyedi et al., 2013), increasing the petal diameter and length are expected. In line with the current research, previously, the positive impact of calcium on improving the morphological traits of rose and gerbera has been reported (Abdolmaleki et al., 2015; Aghdam et al., 2019).

Fresh weight, as one of the key qualitative factors, determines the freshness, form, and longevity of cut flowers (Chanasut et al., 2003). The notably higher fresh weight of rose cultivars suggests that calcium enhances the relative water content of petals and supports greater floret weight (Ahmad & Rab, 2020). The effect of calcium in increasing the fresh weight of gladiolus (Ahmad & Rab, 2020) and rose (Mortazavi et al., 2007) has been documented. In addition, the application of calcium chloride has been found effective in preserving fresh weight and maintaining the quality of Berberis vulgaris (Moradinezhad et al., 2018). Moreover, A comparable response in the two rose cultivars with different genetic backgrounds indicates that calcium exerts a uniform effect on vase life extension and the regulation of senescence (Torre et al., 1999).

Flower longevity is considered a critical parameter for evaluating floral quality. Research has demonstrated that calcium plays a crucial role in slowing the senescence of plant tissues (Ferguson & Drobak, 1988), particularly affecting rose petals (Banijamali et al., 2018). The senescence process in roses is influenced by multiple biochemical and physiological factors, including the integrity and composition of cell membrane proteins and phospholipids, ethylene production, and ATPase enzyme activity (Faust & Klein, 1974; Ferguson & Drobak, 1988). Maintaining optimal calcium levels in plant tissues can stabilize these cellular components, thereby slowing down petal wilting and extending overall flower longevity. It is worthy to note that calcium plays an important role in above mentioned factors. So, the application of calcium results in retarding floral senescence, preservation of membrane integrity, reducing the rate of reactive oxygen species (ROS), decrease in free spaces of floral tissue, increasing cell turgor pressure and promoting water saving that all happened during aging (Abdolmaleki et al., 2015; Ahmad & Rab, 2019; Khalaj et al., 2021). The positive role of calcium in prolonging the vase life of cut flowers has been documented in roses (Banijamali et al., 2018) and gladiolus (Khalaj et al., 2021). Likewise, foliar calcium treatments have been reported to slow down senescence in gerbera (Javad et al., 2011). Consistent with the present study, previous research also indicated that spraying calcium nitrate effectively prolongs the longevity of cut gladiolus flowers (Ahmad & Rab, 2019).

The observed enhancement in leaf greening index following foliar and fertigation treatments with calcium nitrate is attributed to calcium's essential function in chlorophyll synthesis. Calcium activates multiple enzymatic pathways and prevents their inhibition by reducing soluble oxalate accumulation in leaves (Al-Ibraheemi et al., 2021), thus supporting chlorophyll production. Comparable beneficial effects of calcium on chlorophyll content have been reported in rose, Moss Portulaca grandiflora, and gerbera, which aligns with the findings of the current study (Al-Ibraheemi et al., 2021; Roosta et al., 2024). Furthermore, calcium promotes plant growth by enhancing the uptake of key mineral nutrients, such as nitrogen and magnesium, which are integral components of chlorophyll molecules (Roosta et al., 2024). These mechanisms collectively explain the improved leaf greening observed under calcium nitrate treatments.

Application of calcium nitrate increased anthocyanin content in two rose cultivars under hydroponic system. The rise in anthocyanin content due to the calcium consumption can be attributed to the fact that the calcium plays a key role in signalling between cells, which in turn resulted in carbohydrate absorption in the cell and the synthesis of anthocyanin (Mohammadbagheri & Naderi, 2017). The researchers reported that the positive impact of calcium

treatment on enzyme activity of phenylalanine ammonia lyase (PAL) led to an enhancement of anthocyanin biosynthesis (Li et al., 2002).

Spraying calcium nitrate can exert a positive effect on improving the antioxidant enzyme activity of SOD, POD, and CAT by providing a positive charge to scavenge free radicals, decreasing the negative impacts of free radicals, and assisting in cellular antioxidant activity (Alikhani et al., 2021). Based on available references, the application of calcium has resulted in increasing antioxidant (enzymatic/nonenzymatic) reactions (Ehsanimehr et al., 2024), which was in concurrent with the results of this research. In earlier researches, plant resistance to environmental stress caused by calcium application have been reported (Jiang & Huang 2001). Moreover, calcium acts as a secondary messenger (calmodulin proteins) provides activating antioxidant systems to resist stem bending (Alikhani et al., 2021).

The application of foliar spraying results in the enhancement of plant growth and yield through intensifying nutrient absorption and production in plants. Importantly, our study demonstrates that foliar Ca can partially compensate for reduced Ca in the nutrient solution under soilless cultivation, leading to positive effects on mineral content, antioxidant defense, and postharvest longevity. These findings provide practical insights for optimizing calcium management in hydroponic rose production. The calcium levels in the roots were significantly greater than those observed in the petals and leaves of both cultivars. This pattern could be attributed to the availability of calcium to the roots in the nutrient solution (Sobczak et al., 2024). Due to the proximity of the leaves to the roots, the content of calcium in leaves was much more than that of in petals. It seems that the restricted mobility of the calcium ion, and the dependence of calcium translocation to its levels in the solution and the process of transpiration, roots can accumulate more calcium than leaves which transpired more than other organs (Albino-Garduño et al., 2007; Sobczak et al., 2024). Additionally, the application method of calcium nitrate through fertigation is the other reason of the highest calcium content in roots compared with other structures. Sairam et al. (2011) found that higher calcium concentration in petals was followed from the calcium application via holding solution, which was not concurred with the current study that the highest calcium content recorded in petals through foliar applied calcium. Regarding to Mulder's chart, calcium has a reverse impacts on the absorption of Fe, Mn, and Zn (Liu et al., 2023). In our study, the impacts were adverse depending on the application method, cultivar, and the organ namely an increase in the Fe and Mn contents for the plants sprayed with calcium nitrate and a decrease in the Fe, Mn, and Zn contents for the plants fertigated with this fertilizer. However, Zn content decreased in leaf of plants sprayed with calcium nitrate.

Conclusion

Comparison of fertigation and foliar spraying of calcium nitrate on flower quality highlights the superior role of foliar application in enhancing flower longevity. Calcium application positively influenced growth parameters and antioxidant enzyme activities in both rose cultivars. Floral traits, including flower size, leaf greening index, calcium content in leaves and petals, as well as root and leaf micronutrients (Mn, Fe, and Zn), were significantly enhanced in response to foliar calcium nitrate treatment, even when only 10% of the recommended Ca was supplied via the nutrient solution This application also improved the relative fresh weight of petals in both cultivars, reducing floral bending and thereby extending flower longevity. Meanwhile, root length, root volume, and root calcium concentration reached their highest values across both cultivars. Notably, foliar spraying of calcium nitrate substantially increased the activities of antioxidant enzymes POD, SOD, and CAT in the "Samurai" cultivar. Collectively, these findings indicate that foliar application of calcium nitrate, even when supplying only 10% of the recommended fertilizer via nutrient solution, effectively increases calcium accumulation in petals, enhances vase life, and maintains postharvest freshness and consumer acceptability in rose cultivars. Limitations of this study include the use of only two cultivars, a single Ca concentration, and one greenhouse location. Future research should explore different Ca levels, combined nutrient strategies, and interactions with postharvest treatments to optimize vase life and quality in soilless rose cultivation.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

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