Biochemical Contents, Spike Quality and Postharvest Longevity of Gladiolus in Response to Foliar Application of Calcium

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Summary

The majority of gladiolus cultivars are sensitive to spike bending and postharvest longevity. The field trial was performed to evaluate the use of various calcium fertilizers on some biochemical attributes, growth and postharvest trading criteria of *Gladiolus grandiflorum* L. cv 'Rose supreme'. During two consecutive years, two Ca sources at different levels (0, 0.3, 0.6, 0.9, 1.2 and 1.5 g L^{-1}) were sprayed on the gladiolus plants when the tip of flowering stem was observed and 10 days after the appearance. The results in both years revealed that, in comparison to non-Ca sprayed plants, total soluble carbohydrates and total protein content were increased by ~74.2% and 217% in the plants at 1.5 g L^{-1} Ca applied, whereas the highest total anthocyanin and proline content were observed at 1.2 g L^{-1} Ca application. The membrane stability augmented as the Ca fertilizer level increased. Antioxidant enzymes, namely POD and SOD in petals remained at the highest with 1.2 g L^{-1} Ca. Ca applied at 1.2 g L^{-1} imparted greater useful influence on spike strength and vase life longevity (by ~21.5 days) than the other concentrations. Ca $(NO_3)_2$ fertilizer increased flower longevity by 11/7% longer than did . However, corm features have not been significantly affected by any Ca sources and levels. Among various biochemicals and floral features, antioxidative enzymes were revealed to have a positive correlation with vase life. Path analysis revealed that only total protein and Ca content recorded the highest magnitude (3.417 and 1.363, respectively) of positive direct effect on vase life. Proline content and number of florets per spike had strong negative direct effects of -3.068 and -2.580, respectively, on vase life. Our results suggest that Ca prolongs the vase life through improving antioxidative defense system and some osmolytes.

Key words

antioxidative enzyme, Ca nutrient, carbohydrate, gladiolus, vase life

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Introduction

Gladiolus as an ornamental geophyte is used for herbaceous border, rockery, bed, pot and particularly for cut flowers that are in high demand in global trade (Ahmad et al., 2016). However, despite their diverse colors, shapes and ease of culture, gladioli often have short longevity of postharvest due to rapid petal decoloring and wilting (Saeed et al., 2013). The main features that determine the quality and marketability of the gladiolus cut flowers are listed as spike size, flower color, the number and size of florets per spike and vase life (Singh et al., 2008). Meanwhile, in trading industry of gladiolus, higher stem elongation leads to higher stem bending during vase life. It would be a great challenge especially in countries, i.e. Iran, where the people tend to buy gladiolus cut flower with larger flowering stem containing more florets number. To cope with the problem, it seems that the attention to the nutrients strengthening stem cell wall is a critical way. It has been reported that the preharvest nutritional treatments are much more efficient in the shelf life than the postharvest ones (Kou et al., 2015).

Petal wilting, decoloring and senescing generally result from the production of reactive oxygen species (ROS) in plants. Plant cells contain both enzymatic (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), etc.) and non-enzymatic antioxidants (proline, carotenoids, glutathione, etc.) for protection against ROS (Liang et al., 2009).

Calcium is an essential nutrient that contributes to the stability of the surface of cell membrane, the modulation of cells pH and prevents the solute leakage from plant cell cytoplasm (El-Beltagi and Mohamed, 2013). It plays a crucial role in the division and the elongation of plant cells, the translocation of carbohydrate and the metabolism of nitrogen (Khursheda et al., 2015). However, because of low solubility, calcium compounds already existing in soil cannot be sufficiently absorbed by roots, and therefore they may not satisfy the plants needs (Larbi et al., 2020). Moreover, calcium circulation is usually low inside the plants. Calcium-contained fertilizers such as calcium nitrate and calcium chloride are inexpensive and safe fertilizers that have usually been applied to agricultural products (Akladious and Mohamed, 2018). Specifically, the use of $CaCl_2$ in fertilizing is known to involve in maintenance of the function and structure of plant cell membranes (Jiang and Huang, 2001), to influence photosynthesis efficiency and activity of antioxidant enzymes (Liang et al., 2009) and to affect biochemical contents of fruits (Ramezanian et al., 2009). Calcium nitrate has been indicated to affect plant growth such as dry matter and tuber weight (Hamdi et al., 2015).

The application of cost effective and environment-friendly approaches is necessary for enhancing both growth quality and postharvest longevity of cut flowers. In recent years, the production of field grown gladiolus cut flowers has become dramatically popular in Mahallat region of Iran. Therefore, in the present study, a field trial was conducted over two years to monitor the effects of preharvest foliar application of two different calcium sources, calcium chloride and calcium nitrate, on phytochemical properties, growth and vase life trading criteria of *Gladiolus grandiflorum* L. cv 'Rose supreme'. Path analysis was carried out using the procedure suggested by Dewey and Lu (1959), which has been used to quantify a perceived biological relationship through partitioning of correlation coefficients into direct and indirect effects.

Materials and Methods

Experimental Site Description

The experiment was performed during two consecutive years, 2021 and 2022, at the Ornamental Plants Research Center (OPRC) in Mahallat (33° 54' N, 50° 27' E, elevation of 1747 m), Iran. The climate is a local steppe climate with an annual precipitation of 175 mm and relative humidity (RH) of around 50%. Annual temperature varies from -5 to 15 °C in winter and 15 to 35 °C in summer. Physicochemical soil analysis has indicated that it is Xeric Torriorthents classification with a sandy loam texture (about 22.9% silt, 61% sand and 1% clay). The irrigation water and top soil (30 cm in depth) properties were analyzed (Table 1 and 2).

Plant Material and Treatments

The corms used in this study were pure strain of gladiolus (*G. grandiflorum* L. cv 'Rose supreme') obtained from OPRC in Mahallat. Corms were surface sterilized with sodium hypochlorite (0.5% V/V) for 10 min, followed by dipping in a copper sulphate solution (5%) as a fungicide for 3 min and rinsing 3 times with distilled water. The planting of corms was conducted at distances of 15 × 20 cm. Irrigation was carried out at 14 days interval. The applied treatments were calcium as $Ca(NO_3)_2$ (98.5%, Merck) and calcium as $CaCl_2$-$2H_2O$ (99%, Merck) at concentrations of 0.3, 0.6, 0.9, 1.2, and 1.5 g L^{-1}. These treatments were foliar-applied twice, when the apex of flowering stem appeared and 10 days after the appearance. As nonionic tensioactive, Tween-20 (2 mL L^{-1}) was added to all treatment solutions to increase the absorption of calcium compounds. All of foliar application of the solutions was done in the morning (7– 9 a.m.). Nitrogen was partitioned into three applications (preplanting, after 30 and 60 days of planting). Other nutrients were given just in planting and the potassium sources were applied to the soil 30 days before planting. For each treatment three replications (10 plants in each replication) were used.

When the lowermost 2-3 florets commenced to show color, the spikes of the plant were harvested and immediately transported to a water-filled bucket. The stems basal part was cut with 1-2 bract leaves below the florets, and then they were kept at ambient storage.

Growth and Vase Life Features

At harvesting time, commercial growth features were recorded. They included the spike length and strength, the number of florets per spike, corm fresh and dry weight, corm diameter, and the number of cormlet. The spike strength was also measured based on the deviation of stem from straight line. The actual vase life was considered as the duration between the opening of first floret and the wilting of the fifth floret from bottom (Beura and Singh, 2001). The unopening florets (%) were determined simultaneously with actual vase life measurement.

The calcium content in plant tissues was measured using titration against versene solution (Na-EDTA) based on the procedure of Kinzel (1989) and expressed as percent of dry weight (DW).

Table 1. Analysis of water used for irrigation of *Gladiolus grandiflorum* L. cv.'Rose supreme'

SAR	EC (µmhos cm^{-1})	pH	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Sum Anions	$Ca^{2+} + Mg^{2+}$	Na^+	Sum Cation	Sample
0.79	877	7.38	0	6.13	1.5	1.12	8.75	7.18	1.5	8.68	water

Table 2. Analysis of field soil where *Gladiolus grandiflorum* L. cv.'Rose supreme' was cultivated

EC	pH	T.N.V	Organic contents	N	P	K	Fe	Zn	Mn	Cu	B	Sand	Silt	Clay	Texture
dS/M				%			Mg kg^{-1}					%			
1.24	7.85	38	0.34	0.034	6.2	160	3.1	0.58	4.74	0.8	0.55	61	22.9	16.1	Sandy loam

Biochemical Analyses

Total Soluble Carbohydrate

Total soluble carbohydrate (TSC) was determined by the colorimetric test according to DuBois et al. (1956) with some modifications. Briefly, TSC was extracted from 0.5 g of petal sample through extracting once with 8 mL of 80% methanol at 90 °C and then it was extracted again with 8 mL double-distilled water at 65 °C. The extractions were applied for determining the TSC quantified at 620 nm absorption.

Enzyme Activity

For enzyme extraction, 0.5 g of fine crushed and powdered petal samples were placed in a microtube containing 2 mL of phosphate buffer (50 mM, pH 7.0) including 1% PVP and 1 mM EDTA. The mixture was centrifuged at 13,000 × g for 15 min at 4 °C, and the supernatant was used for assay of the following enzyme activity.

The assay of peroxidase (POD, EC: 1.11.1.7) activity was accomplished as described by (Hemeda and Klein, 1990). The reaction mixture of 100 mL contained 10 mL of 0.3% H_2O_2, 10 mL of 1% guaiacol (v/v) and 80 mL of phosphate buffer (50 mM, pH 7.0). Enzyme extract (75 µL) was added to the reaction mixture in the final volume of 3 mL. The absorbance increase resulting from guaiacol oxidation (extinction coefficient 26.6 mM^{-1} cm^{-1}) was monitored at 470 nm. Finally, it was expressed as U g^{-1} protein.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was estimated based on the method of Flohé and Otting F. (1984) which determines spectrophotometrically the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. One unit of enzyme activity was known as the amount of SOD needed for producing a 50% inhibition of NBT reduction and the specific activity of SOD was expressed as U g^{-1} protein.

Total Protein

Total protein (TP) was measured according to the method of (Bradford, 1976). Crystalline bovine serum albumin was used as a reference.

Lipid Peroxidation

As an indicator of membrane integrity, malondialdehide (MDA) contents were measured according to the procedure of (Heath and Packer, 1968). The petal sample (0.25 g) was placed in 5% trichloroacetic acid (TCA), and then was filtered through Whatman No. 1 filter paper . The extract reaction was done with 15% TCA containing 0.5% thiobarbituric acid, followed by incubating at 70 °C for 30 min. Afterwards, the solution was placed immediately into an ice bath. The absorbance at 600 and 532 nm was recorded. The concentration of MDA was calculated by an extinction coefficient of 1.55 mM^{-1} cm^{-1} as follows: MDA content = [(OD_{532} – OD_{600}) × 2 mL × (total volume of extract × 1 mL)] / (1.55 × 10−1 × sample weight). Data were considered as nM g^{-1} FW.

Proline Content

The determination of proline content was done based on the procedure of (Bates et al., 1973). Briefly, 0.5 g of fresh petal sample

was homogenized in 10 mL of sulfosalicylic acid (3%). After centrifuging at 12,000 × g, the supernatant (2.0 mL) was added to 2 mL glacial acetic acid and 2 mL acid ninhydrin in a test tube. The test tubes were heated for 60 min at 100 °C in a hot water bath. Afterwards, they were immediately translocated to an ice bath to cease the reaction. To each of the test tubes, toluene (4.0 mL) was added and subsequently stirred strongly for 10–20 s. The absorbance was read against reagent blank at 520 nm using a VIS/UV spectrophotometer (Shimadzu, UV-1201). Proline content was expressed as μM g^{-1} FW.

Total Anthocyanin

The procedure of Zhishen et al. (1999) was applied for measurement of total anthocyanin (TA) in the petal samples. The absorbance at 520 and 700 nm was measured, and results were expressed as mg cyanidin-3-glucoside 100 g^{-1} FW.

Statistical Analyses

All field treatments and laboratory measurements were done in triplicate. Statistical analysis was made by one-way analysis of variance (ANOVA) followed by a Tukey's test comparison means test at $P < 0.05$ significance level in SAS software (Version 9.1). Correlation analysis was carried out in SPSS (Version 22). Path analysis was performed as described by using Path2 software.

Results

Growth Characteristics and Postharvest Longevity

The results showed that there was a significant difference between the type and concentration of calcium fertilizer regarding flower, corm and vase life criteria. However, the corm-related traits such as corm number, size and dry and fresh weight, were not significantly affected by the type of calcium sources. Moreover, different concentrations of both types of calcium sources had different effects on the studied traits. As shown in Table 3, the highest spike strength (17.56 cm), and the maximum unopening florets (5.56%) were obtained in plants treated with 1.2 and 1.5 g L^{-1} calcium, respectively. However, as Ca concentrations increased in fertilizer sources, Ca content increased in leaves, too, so that the highest Ca content was observed in 1.5 g L^{-1} Ca-treated plants. Meanwhile, the spike length of 1.5 g L^{-1} Ca-treated plants was 10% higher than that of control plants. The results exhibited that, among the flower-related traits, the number of florets per spike was not significantly affected by Ca fertilizer. Similarly, some corm-related characteristics such as corm number and diameter did not change as Ca concentration increased. Moreover, at Ca concentrations higher than 6 g L^{-1}, no significant alteration occurred in corm weight (Table 4). As a critical feature, vase life of gladiolus plants was considerably affected by Ca fertilizer. So, it was ~ 29.5% higher in the plants sprayed with 1.2 g L^{-1} Ca than it was in control plants.

Concerning the type of Ca fertilizer source, calcium nitrate increased flower longevity by 11/7% longer than did calcium chloride. Calcium content in the petals and corms sprayed with 1.5 g L^{-1} Ca in 2021 increased by 30% and ~16.5% as compared to that in non-Ca treated ones. In all, as a comparative point of view in 2021 and 2022, no significant changes were observed among either corm-related traits or flower quality and postharvest criteria. Regarding corm-related measurements, both types of Ca fertilizer sources had statistically similar effects.

Table 3. Effect of various calcium concentrations and types on flower characteristics and vase life of *Gladiolus grandiflorum* L. cv.'Rose supreme'

Ca concentrations (g L^{-1})	Number of floret per spike		Spike length (cm)		Spike strength (cm)		Unopening florets (%)		Ca in petal (%)		Vase life (day)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
0	16^{a}	17^{a}	52.17^{b}	53.90^{b}	11.88^{e}	11.78^{e}	2.20^{e}	1.90^{e}	0.60^{e}	0.70^{c}	16.73^{d}	16.63^{d}
0.3	18^{a}	17^{a}	53.50^{b}	53.84^{b}	13.31^{d}	12.48^{d}	2.38^{e}	2.00^{e}	0.67^{bc}	0.77^{bc}	17.85^{cd}	16.85^{d}
0.6	16^{a}	18^{a}	52.56^{b}	52.19^{b}	14.80^{c}	14.65^{ab}	2.75^{c}	2.12^{c}	0.68^{b}	0.78^{b}	19.56^{ab}	19.97^{a}
0.9	16^{a}	17^{a}	53.11^{b}	53.17^{b}	15.45^{ab}	14.85^{ab}	3.46^{b}	2.92^{b}	0.71^{ab}	0.81^{ab}	21.00^{a}	21.11^{a}
1.2	17^{a}	17^{a}	53.61^{b}	54.00^{b}	17.56^{a}	17.46^{a}	3.90^{b}	3.60^{b}	0.75^{a}	0.85^{a}	21.16^{a}	21.51^{a}
1.5	17^{a}	16^{a}	57.54^{a}	56.90^{a}	16.55^{ab}	16.00^{ab}	5.56^{a}	4.56^{a}	0.78^{a}	0.88^{a}	18.46^{bc}	18.56^{bc}
Ca type												
Ca $(NO_3)_2$	18^{a}	17^{a}	54.19^{a}	55.12^{a}	14.17^{b}	14.19^{b}	2.97^{b}	1.87^{b}	0.72^{a}	0.72^{a}	20.20^{a}	21.20^{a}
$CaCl_2$-$2H_2O$	17^{a}	18^{a}	54.89^{a}	54.90^{a}	15.67^{a}	14.97^{a}	3.78^{a}	4.72^{a}	0.68^{b}	0.68^{b}	18.08^{b}	19.18^{b}

Note: Means in each column followed by the same letters are not significantly different by Tukey's test at $P < 0.01$ level

Phytochemical Features

As shown in Table 5, petal biochemical contents were significantly altered after foliar application of Ca. In comparison to non-Ca sprayed plants, TSC and total protein content were increased by ~74.2% and 217% in the plants at the maximum Ca applied, 1.5 g L^{-1}, whereas TA and proline content were highest with 1.2 g L^{-1} Ca application. The highest membrane permeability, as indicated by lipid peroxidation, occurred in the plants that received no Ca fertilizer. On the other hand, lipid peroxidation reduced by ~70.7% and 68% in petal tissues at 1.2 g L^{-1} Ca level, in 2021 and 2022, respectively, followed by 1.5 g L^{-1} Ca level.

The results put forth an increase in antioxidative enzymes followed by increase in Ca concentration up to 1.2 g L^{-1}. The activity of antioxidative enzymes such as POD and SOD, were at their highest values by the foliar application of Ca at 1.2 g L^{-1}. Moreover, high concentration of Ca, 1.5 g L^{-1}, did not encourage higher antioxidative enzymes. The least POD and SOD activities, 0.21 and 26.19 U g^{-1} protein, were recorded with non-Ca treated plants. The results in both years (2021 and 2022) showed no significant change in measured petal biochemicals by Ca application over the control treatment. However, calcium nitrate fertilizer enhanced significantly all the biochemicals more than calcium chloride did.

Correlation and Path Analysis

The results showed that petal Ca content had significantly a positive correlation with spike strength, proline, TA, total protein and TSC, while it had a negative correlation with MDA, as indicator of lipid peroxidation. Among various biochemicals and floral features, antioxidative enzymes, viz., POD and SOD, were revealed to have a positive correlation with vase life (Table 6). On the other hand, spike length and number of florets per spike had a negative correlation with vase life. The correlation coefficient of petal Ca content with vase life was positive and non-significant, whereas it revealed a strong positive and highly significant relationship with TSC (0.973) and TA (0.972). As shown in Table 7, path coefficient analysis revealed that only total protein and Ca content recorded the highest magnitude (3.417 and 1.363, respectively) of positive direct effect on vase life. Proline content and number of florets per spike had strong negative direct effects of -3.068 and -2.580, respectively, on vase life. Similarly, MDA exhibited the highest negative indirect effect of -3.336 via total protein on vase life. A strong positive indirect effect of Ca content on vase life was recorded via total protein, while the highest indirect effects of antioxidative enzymes, POD and SOD, on vase life were observed via number of florets per spike. A strong negative indirect effect of TSC (−2.654), POD (-1.234), SOD (-1.976), TP (-2.955), TA (-2.844), spike strength (-2.884) and Ca content (-2.912) were noticed through proline content on vase life. The influence of all included variables on vase life was 4.189 and Ca content contributed 14.7% to vase life.

Table 4. Effect of various calcium concentrations and types on corm features of *Gladiolus grandiflorum* L. cv. 'Rose supreme'

Ca concentrations (g L^{-1})	Ca in corm (%)		Corm diameter (cm)		Corm number		Corm fresh weight(g)		Corm dry weight(g)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
0	0.30^{d}	0.26^{d}	10.71^{a}	12.71^{a}	4.86^{a}	5.87^{a}	52.96^{c}	53.86^{c}	13.55^{c}	14.95^{c}
0.3	0.31^{c}	0.31^{c}	11.35^{a}	12.35^{a}	4.93^{a}	5.91^{a}	57.21^{bc}	56.22^{bc}	15.73^{bc}	18.83^{b}
0.6	0.33^{b}	0.41^{b}	11.68^{a}	11.48^{a}	5.15^{a}	5.82^{a}	61.80^{abc}	61.86^{ab}	16.98^{bc}	18.98^{b}
0.9	0.34^{ab}	0.44^{ab}	11.90^{a}	11.80^{a}	5.03^{a}	6.23^{a}	65.43^{ab}	71.43^{a}	18.93^{ab}	22.93^{a}
1.2	0.34^{a}	0.43^{a}	11.95^{a}	12.95^{a}	5.20^{a}	6.20^{a}	71.70^{a}	72.00^{a}	22.58^{a}	24.67^{a}
1.5	0.35^{a}	0.45^{a}	12.10^{a}	12.08^{a}	5.56^{a}	6.12^{a}	72.60^{a}	72.50^{a}	22.75^{a}	24.45^{a}
Ca type										
Ca $(NO_3)_2$	0.34^{a}	0.36^{a}	12.02^{a}	12.34^{a}	5.21^{a}	5.01^{a}	66.50^{a}	59.90^{a}	19.43^{a}	18.76^{a}
$CaCl_2$-$2H_2O$	0.32^{b}	0.36^{a}	11.20^{a}	12.43^{a}	3.93^{a}	4.91^{a}	60.73^{a}	61.53^{a}	17.40^{a}	19.40^{a}

Note: Means in each column followed by the same letters are not significantly different by Tukey's test at $P < 0.01$ level

Table 5. Effect of various calcium concentrations and types on biochemical attributes of *Gladiolus grandiflorum* L. cv. 'Rose supreme'

Ca concentrations ($g\ L^{-1}$)	Total soluble carbohydrate ($mg\ g^{-1}$ FW)		Peroxidase ($U\ g^{-1}$ protein)		Superoxide dismutase ($U\ g^{-1}$ protein)		Total soluble protein ($mg\ g^{-1}$ FW)		Lipid peroxidation ($nM\ g^{-1}$ FW)		Proline content ($\mu M\ g^{-1}$ FW)		Total anthocyanin (mg cyanidin-3-glucoside 100 g^{-1} FW)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
0	14.57^{d}	15.98^{d}	0.21e	0.27e	26.19^{d}	27.20^{d}	11.10^{e}	12.14^{d}	70.19^{a}	69.12^{a}	0.81^{e}	0.92^{e}	2.53^{c}	3.11^{c}
0.3	20.79^{c}	21.56^{c}	0.39^{d}	0.34^{d}	27.93^{c}	28.94^{c}	11.13^{e}	12.13^{d}	69.89^{a}	67.85^{a}	0.92^{d}	0.94^{e}	2.90^{c}	3.15^{c}
0.6	20.89^{c}	21.86^{c}	0.64^{b}	0.61^{c}	32.16^{b}	33.17^{b}	14.65^{d}	12.65^{d}	64.12^{b}	59.16^{b}	0.94^{d}	1.16^{d}	3.45^{b}	3.98^{b}
0.9	22.98^{b}	22.76^{b}	0.80^{a}	0.83^{a}	34.12^{a}	35.13^{a}	28.60^{c}	29.68^{c}	55.76^{c}	58.96^{b}	1.23^{c}	1.45^{c}	3.60^{b}	4.21^{b}
1.2	24.77^{a}	26.07^{a}	0.54^{c}	0.84^{a}	32.17^{b}	35.11^{a}	34.12^{b}	34.13^{b}	40.12^{d}	40.18^{c}	1.45^{b}	1.70^{b}	4.23^{a}	4.54^{a}
1.5	25.39^{a}	26.43^{a}	0.52^{c}	0.72^{b}	32.15^{b}	33.16^{b}	35.21^{a}	35.91^{a}	41.24^{d}	42.02^{c}	1.67^{a}	1.97^{a}	4.19^{a}	4.69^{a}
Ca type														
$Ca(NO_3)_2$	23.12^{a}	25.14^{a}	0.69^{a}	0.63^{a}	33.78^{a}	34.65^{a}	29.45^{a}	29.41^{a}	53.12^{a}	52.16^{a}	1.56^{a}	1.66^{a}	4.14^{a}	4.79^{a}
$CaCl_2$-$2H_2O$	21.09^{b}	22.39^{b}	0.57^{b}	0.61^{b}	32.01^{b}	33.01^{b}	23.34^{b}	28.95^{a}	54.99^{b}	57.09^{b}	1.49^{a}	1.39^{b}	4.09^{a}	3.59^{b}

Note: Means in each column followed by the same letters are not significantly different by Tukey's test at $P < 0.01$ level

Table 6. Correlation between vase life components in *Gladiolus grandiflorum* L. cv. 'Rose supreme'

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. TSC	1	0.665ns	0.803*	0.839*	-0.842*	0.865*	0.935**	-0.524ns	0.670ns	0.945**	0.798*	0.973**	0.694ns
2. POD		1	0.948**	0.517ns	-0.416ns	0.402ns	0.630ns	-0.933**	0.104ns	0.622ns	0.356ns	0.549ns	0.849*
3.SOD			1	0.736*	-0.671ns	0.644ns	0.833*	-0.814*	0.315ns	0.822*	0.599ns	0.748ns	0.871*
4. TP				1	-0.976**	0.963**	0.921**	-0.224ns	0.660ns	0.918**	0.894**	0.911**	0.642ns
5. MDA					1	-0.974**	-0.943**	0.152ns	-0.706ns	-0.936**	-0.915**	-0.934**	-0.577ns
6. Proline						1	0.927**	-0.148	0.830*	0.940**	0.969**	0.949**	0.476ns
7. TA							1	-0.443ns	0.682ns	0.996**	0.888**	0.972**	0.683ns
8. NFS								1	0.030ns	-0.438ns	-0.134ns	-0.365ns	-0.706ns
9. SL									1	0.735ns	0.908**	0.759ns	-0.017ns
10. SS										1	0.913**	0.980**	0.641ns
11. UF											1	0.897**	0.324ns
12. Ca												1	0.616ns
13. VL													1

Note: ns, * and ** are not significant, significant at $P < 0.05$ and $P < 0.01$ level, respectively. TSC: total soluble carbohydrate; POD: peroxidase; SOD: superoxide dismutase; TP: total protein; TA: total anthocyanin; NFS: number of florets per spike; SL: spike length; SS: spike strength; UF: unopening florets; Ca: calcium content in petal; VL: vase life

Table 7. Path analysis showing direct and indirect effects of different characters on vase life of *Gladiolus grandiflorum* L. cv. 'Rose supreme'

	Character	1	2	3	4	5	6	7	8	9	10	Correlation with vase life
1	TSC	**-0.341**	-1.145	-0.403	2.867	1.179	-2.654	-1.217	1.351	-0.279	1.331	0.694ns
2	POD	-0.227	**-1.721**	-0.475	1.766	0.582	-1.234	-0.821	2.406	-0.184	0.751	0.849*
3	SOD	-0.274	-1.632	**-0.501**	2.015	0.940	-1.976	-1.085	2.599	-0.243	1.023	0.871*
4	TP	-0.286	-0.890	-0.369	**3.417**	1.367	-2.955	-1.199	0.577	-0.271	1.246	0.642ns
5	MDA	0,287	0.715	0.336	-3.336	**-1.402**	2.988	1.227	-0.393	0.275	-1.279	-0.577ns
6	Proline	-0.295	-0.692	-0.323	3.291	1.363	**-3.068**	-1.207	0.381	-0.278	1.294	0.476ns
7	TA	-0.319	-1.084	-0.418	3.147	1.321	-2.844	**-1.302**	1.142	-0.294	1.330	0.683ns
8	NFS	0.178	1.605	0.407	-0.766	-0.214	0.454	0.576	**-2.580**	0.129	-0.500	-0.706ns
9	SS	-0.323	-1.071	-0.412	3.137	1.311	-2.884	-1.297	1.129	**-0.295**	1.341	0.641ns
10	Ca	-0.332	-0.945	-0.375	3.113	1.308	-2.912	-1.266	0.941	-0.289	**1.363**	0.616ns
Residual effects =				= 0.07								

Note. Bold numbers are the direct effects ns, * and ** are not significant, significant at $P < 0.05$ and $P < 0.01$ level, respectively. TSC: total soluble carbohydrate; POD: peroxidase; SOD: superoxide dismutase; TP: total protein; MDA: malondialdehyde; TA: total anthocyanin; NFS: number of florets per spike; SS: spike strength; Ca: calcium content in petal

Discussion

In the trading industry of ornamental plants, especially flowering geophytes, the spike bending and petal decoloring have always been a great challenge. To cope with the problem, the attention to the nutrients strengthening spike cell wall and increasing cellular defense system against senescing agents is a crucial strategy. Many researchers have disclosed the role of preharvest nutrients and lack of calcium, an element that is related directly to the cell membrane stability. The field trial was mainly aimed to test the effect of calcium fertilizer types and concentrations on growth, flower features and vase life longevity of gladiolus cut flower. It has been reported that the preharvest nutritional treatments are much more efficient in the shelf life than the postharvest ones. Foliar application of fertilizers is one of the key ways to get available nutrients easily to the plant tissues (Singh et al., 2008).

The results from the present study have indicated that Ca affects flower quality and quantity indices and vase life, but it has no effect on the corm production. The presence of less Ca in corm than of that in leaves after foliar Ca application, can be attributed to the lower mobilization of Ca in plant tissues. Therefore, it seems that the use of Ca fertilizers as foliar application on gladiolus is more useful for spike strengthening than its use as fertigation approaches.

Zubair (2011) has found that K at rates more than 100 Kg ha^{-1} enhances corm production due to its influence on the translocation of newly synthesized photosynthates and their mobilization of stored materials in the mother corm. Moreover, in gladiolus, the use of B at 1.0 Kg ha^{-1} caused the production of more corms with larger size (Halder et al., 2007). Phosphorous at rates higher than 40 Kg ha^{-1} increases the number and size of florets and spike length and induces larger corms (Shaukat et al., 2012). Based on our study, Ca increased osmolytes such as proline and total soluble carbohydrates as well as improved antioxidative systems by increasing in POD, SOD and total anthocyanin. The enhancement of antioxidative enzymes has been proved to increase the longevity of flowers during postharvest. Wang et al. (2017) revealed that $Ce(NO_3)_3$ prolonged the vase life of *Rosa chinensis* Jacq. cut flower through enhancing the antioxidant defense system in the petals and the pigment contents in the calyces. Proline has been indicated to fortify the plant defense system against senescence. Proline content increased during senescence of 'Rialto' Oriental lily cut flower (Rabiza-Świder et al., 2015). It has been also reported to decrease in the wilted petals and abscission percentage of flower as well as increment in postharvest longevity of *Polyanthus tuberosa* cut flowers (Shahzad et al., 2022).

The application of different calcium sources had significantly different effects regarding some growth parameters. The number of florets, stem length and strength are considered as judgments of spike quality. In the present study, the highest spike strength was achieved by 1.2 g L^{-1} Ca treatment. In gladioulus cut flower, greater number of florets per spike enhances the beauty of the spike. In our study, the number of florets per spike has not been affected by Ca fertilizer, while spikes have been remarkably strengthened as Ca concentration increased in the fertilizer solution. Ca is the main constituent of plant cell wall structure as calcium pectate of middle lamella and is involved in cell membrane formation. It can also function in signaling pathways and in defense response by

inducing brassinosteroids (Furio et al., 2020). The cell membrane permeability as indicated by lipid peroxidation was lower in those gladiolus plants containing higher antioxidative enzyme activities. SOD constitutes the first line of defense against reactive oxygen species (ROS) and is one of the most effective components of the antioxidant defense system in plant cells against ROS toxicity. It catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide (Das and Roychoudhury, 2014). On the other hand, POD originated from mitochondria, chloroplasts, endoplasmic reticulum, and plasma membrane can scavenge peroxide radicals and H_2O_2 from cells (Salehi et al., 2012). In our study, the foliar application of Ca, particularly calcium nitrate fertilizer, enhanced the activity of the enzymes. The considerable reduction in lipid peroxidation in 1.2 g L^{-1} Ca-treated gladiolus plants may be attributed to the increase in the enzymes activities. Lower membrane permeability due to lower lipid peroxidation results in preventing the solute leakage from plant cell cytoplasm (El-Beltagi and Mohamed, 2013). Moreover, the gladiolus plants treated with calcium exhibited more anthocyanin. It has been reported that some holding solution such as sucrose prolong the vase-life of sweet pea (*Lathyrus odoratus*) cut flowers through increasing anthocyanin (Elhindi, 2012). The addition of sugar (2%) into the vase solution of oriental lily 'Stargazer' increased petal color intensity through enhancing anthocyanin content and, thereby, it kept the cut flower quality during postharvest (Han, 2003). Both types of Ca fertilizers led to an increase in anthocyanin content in petals. In our study, plants containing higher anthocyanin remained healthy for more days during postharvest storage. The quality of gladiolus spike is mostly recognized by its length and strength. Spike strength is directly related to Ca status of the plant. In contrast, the application of calcium did not change the length of spikes. In all, vase life was increased with increasing concentrations of calcium up to 1.2 g L^{-1}, while further increase in calcium did not increase the vase life of gladiolus spike. White and Broadley (2003) have shown that excessive Ca due to hypo-osmotic shock in plants.

Regarding the type of fertilizers, gladiolus plants treated with calcium nitrate solution allowed the production of higher spike strength in comparison to the other alternative fertilizer source, calcium chloride, as well as greater commercial durability, as compared to other concentrations and control plants.

Path coefficient analysis is a standardized statistical technique of partial regression. It partitions the correlation coefficients into their indirect and direct impacts. Therefore, the contribution of each trait to vase life could be estimated. It has been used in identifying the characteristics that are beneficial as selection criteria for enhancing mango yield (Kumar et al., 2015) and ber genotypes (Islam et al., 2010). According to our path analysis, total protein showed the highest magnitude of positive direct effect on vase life. Moreover, a strong positive and negative indirect effect of Ca content and MDA, respectively, on vase life was recorded through total protein. These results indicate the pivotal role of proteins in gladiolus postharvest. It has been proved that Ca regulates directly the activities of target proteins or through calcium-binding proteins. In plant cells, after binding to Ca^{2+}, calmodulin activates a series of protein kinases and other proteins (Wang et al., 2004). The highly positive direct effect of total protein and Ca content on vase life can indicate the perfect and true relationship between them. The indirect effects of TSC, POD, SOD, TP, TA on spike strength in most characteristics were negative. The indirect effect of Ca content via proline content on vase life refers to the importance of the osmolyte in plant cells. Proline has been reported widely in plant protection against abiotic environmental stresses (Chun et al., 2018). It plays several protective functions including stabilizing cellular structures, enzymes, osmoprotectant, and keeps up redox balance in adverse situation (Meena et al., 2019). Accordingly, one of the promotive effects of Ca nutrition on longevity of gladiolus can be attributed to proline enhancement. Our research determined the correlations and path analysis of vase life and related components to assess their importance as affected by foliar Ca application in successful gladiolus cut flower management. The residual effect through path analysis indicates the contribution of other factors on the vase life of gladiolus cut flower than the studied ones.

Conclusion

The preharvest application of nutrients is the cost effective and environmentally friendly approach for enhancing both growth quality and postharvest longevity of cut flowers. Both types of Ca fertilizer, calcium nitrate and calcium chloride, particularly at 1.2 g L^{-1} concentration have the positive effects on growth, antioxidant compounds, biochemical constitutes and vase life of gladiolus flowers. Meanwhile, the foliar application of calcium nitrate is more useful than calcium chloride for improving postharvest storage durability in gladiolus plants.

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CRediT Authorship Contribution Statement

Alireza Noroozisharaf: Data analysis, Writing, Reviewing. **Mohammad Ali Khalaj:** Methodology, Conceptualization, English editing. **Mehradad Rasouli:** Original draft preparation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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