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Growth, Flowering, and Durability of *Gladiolus* Affected by Foliar Application of Calcium from Different Sources and at Different Doses

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Abstract

Gladiolus is one of the most important cut flowers in the world, especially in Iran. A field experiment was conducted to evaluate the effect of spraying different calcium doses and sources on the quality and quantity of *Gladiolus grandiflorus* cv. Rose Supreme. The experiment was conducted in a factorial randomized complete block design with three replications in two years in 2017-2019. The calcium concentrations were 0, 0.3, 0.6, 0.9, 1.2, and 1.5 g L^{-1} from the sources of $CaCl_2$ and $CaNo_3$. The data revealed that foliar application of calcium doses had significant effects on most quality and quantity growth parameters. Increasing the calcium dose stimulated lower stalk height, flowering stem diameter, spike length, in florescence fresh and dry weight, inflorescence bending, vase life, and leaf calcium concentration. Calcium at a dose of 1.2 g L^{-1} decreased flower stem bending and increased diameter and vase life by 26.5 and 36.7%, respectively compared to the control. Also, pre-harvest foliar application of calcium nitrate at the rate of 1.2 g L^{-1} increased leaf calcium concentration and stem bending, resulting in delaying senescence and therefore extending flower longevity. The experiment faced no limitations. Then, the application of calcium fertilizers improved the quantitative and qualitative characteristics of gladiolus flowers.

Keywords: Pre-harvest, Quality, Quantity, Stem bending, Vase life.

INTRODUCTION

Gladiolus grandiflorus Hort. is one of the most important and popular bulbous cut flowers in Iran (Azimi *et al.,* 2020 a, b). The cultivation of ornamental plants has economic popularity and is expanding in many countries. The United States, the Netherlands, and Japan are the largest producers and consumers of ornamental plants (Gómez-Pérez *et al.,* 2018), where the pot and cut flowers market makes one of the largest industrial sectors.

Nowadays, *Gladiolus* is one of the world's most momentous bulbous ornamental plants and is a cut flower used for bouquets (Ahmad and Rab*,* 2019). Gladiolus is an attractive and splendor flower, which is known as the bulbous queen flower because of its flower spikes with florets of deluxe colors, marvelous shapes, massive form, different sizes, and supreme shelf life (Jabbar *et al.,* 2017). Gladiolus, from the monocot family of Iridaceae, encompasses about 92 genera and more than 260-300 species that are native to sub-Saharan Africa, mostly South Africa but can be grown in western, southern, and eastern parts of Africa as well as in Southern Europe and Asia (Chore *et al.,* 2020). Gladiolus has a specific position as cut flowers both for local use and global export and is classified as one of the top 10 commercial flowers in the world market (Al-Hasnawi *et al.,* 2019). In Iran, cut gladiolus flowers are produced in summer, but the ycan also be produced in autumn and spring if they are cultivated in protected environmental conditions (Azimi, 2019; Azimi *et al.,* 2020).

It is important to improve the quantity, quality, and longevity of cut flowers from harvest to market (Azimi, 2020 a,b) as these qualities have a direct commercial effect on floricultural value. In recent years, many efforts have been made to increase the yield and quality of cut flowers by different methods (Azimi and Banijamali, 2019). In the ornamentals industry, especially cut flowers, unsuitable conditions and insufficient nutrition often impair the quantity and quality of the produced flowers, affecting their economic value (Aghdam *et al.,* 2019).

Calcium (Ca) plays a vital role in plant growth, development, and response to environmental signals (Aghdam *et al.,* 2019; Khalaj and Noroozisharaf, 2020). As an immobile nutrient, Ca is one of the essential minerals for plant growth (Ahmad and Rab, 2019; Marschner, 2012; Khalaj *et al.,* 2017).The deficiency or lack of calcium shows some physiological disorders at plant terminal parts and growing branch heads. Some other calcium disorders are fruit softness, stem bending in cut flowers, and shorter longevity in fruits and cut flowers (Marschner, 2012; Khalaj *et al.,* 2017). Many studies have been conducted to determine Ca uptake, which can be affected by some factors such as climate conditions, evapotranspiration, and nutrients antagonism, which can directly or indirectly affect Ca absorption and subsequently influence flower yield and longevity (Marschner, 2012; Banijamali *et al.,* 2018). Ca accumulation in plants facilitates pectin polymers' linkage to modify mechanical stem stability in line with decreasing stem bending and increasing flower vase lives (Marschner, 2012; Khalaj *et al.,* 2017). Because of Ca immobility in plants, the foliar application can be helpful to increase its uptake. There are some reports about the effect of pre-harvest Ca sprays on cut flowers' parameters (Belge *et al.,* 2017; Banijamali *et al.,* 2018).

The foliar application of calcium nitrate and calcium chloride increased Ca absorption by the shoot (leaves and stem tissues), which had a direct effect on the growth parameters and vase life of the cut flowers and fruits (Buchanan and Gruissem, 2015; Aghdam *et al.,* 2019).

Exogenous Ca treatments mainly aim to adjust the retention of cell wall integrity, modify gene expression, and improve plant growth and development (Arfaoui *et al.,* 2016; Michailidis *et al.,* 2017). Ca treatment increased stem diameter and stem firmness in *Gerbera jamesonii*

Bolus Ex Hookerf. (Khalaj and Noroozisharaf, 2020; Combrink, 2018). Moreover, exogenous Ca treatments delayed gerbera stem bending (*Gerbera jamesonii* cv. Tamara), which might be due to the coupling of pectin molecules, resulting in more cell wall hardness (Khalaj *et al.,* 2017). Furthermore, a significant increase in the mechanical bending of inflorescence stems was observed in *Paeonia lactiflora* after Ca treatment, which might be due to the changes in cell wall fractions (Zhao *et al.,* 2019).

The present study aimed to evaluate the effect of pre harvest foliar application of Ca at different rates and from different sources on the quantitative and qualitative parameters and postharvest mechanical bending of cut gladiolus inflorescence.

MATERIALSAND METHODS

A field experiment was carried out at the Ornamental Plants Research Center (OPRC) in Mahallat, Iran (33^{o} 54' N, 50^{o} 27' E, elevation 1747 m). The experiment was based on a randomized complete block design with six calcium doses of 0, 0.3, 0.6, 0.9, 1.2, and 1.5 g L^{-1} supplied from $CaCl_2$ and $CaNO_3$ with three replicates in 2017-2019.

Uniform-sized (10-12 cm circumference) *Gladiolus* corms (*Gladiolus grandiflorus* cv. Rose Supreme) were planted in plots (each 2 m^2) spaced by 20 × 15 cm. Foliar fertilizers were sprayed twice, first after inflorescence emergence and second 10 days later. All the cultural operations (fertilization, irrigation, and control) were conducted similarly in all plots. The soil was analyzed for physicochemical properties. The soil texture was sandy loam (22.9% silt, 61% sand, and 16.1% clay) and it was classified as xeric. The soil organic C, total N, P, and K contents were 0.34%, 0.03%, 6.2, and 160 mg kg^{-1}, respectively. The total Fe, Zn, Mn, Cu, and B concentrations were 3.14, 0.57, 4.74, 0.8, and 0.55 mg kg^{-1}, respectively. Also, the soil pH and EC were 7.8 and 1.2 dS m^{-1}, respectively.

Each plot was treated with ammonium nitrate (NH_4NO_3) as a nitrogen source, which was applied in three doses. The first was applied when the corms were planted, and the second and third doses were applied 30 and 60 days later, respectively. A basal application of triple super phosphate [$Ca(H_2PO_4)_2$. x H_2O, 200 kg ha^{-1}] as phosphorus and potassium sulfate (K_2SO_4) as potassium sources were added to the field at the rates of 200 and 360 kg ha^{-1}, respectively. Magnesium sulfate ($MgSO_4.H_2O$, 100 kg ha^{-1}), copper sulfate ($CuSO_4.5H_2O$, 20 kg ha^{-1}), manganese sulfate ($MnSO_4.4H_2O$, 40 kg ha^{-1}), iron chelate (Fe EDDHA, 20 kg ha^{-1}), zinc sulfate ($ZnSO_4.H_2O$, 36 kg ha^{-1}) and boric acid (H_3BO_3, 40 kg ha^{-1}) were also added before planting the corms.

When the first florets of each spike showed pink color, 15 gladiolus spikes were harvested from each plot to evaluate the qualitative and quantitative properties of gladiolus cut spikes. At harvest time in August, the qualitative and quantitative characteristics that were determined included flower stalk height, flowering stem diameter, spike length, inflorescence wet and dry weight, and inflorescence bending. Seven days later, flower stem bending from the straight line, vase life, and leaf calcium concentration were determined. All plant tissue samples used for chemical analysis were initially washed with distilled water, dried at 65˚C until reaching a constant weight, and ground. The extraction of Ca from the plant tissue was performed using wet digestion of the samples with a nitric acid per chloric acid 4: 1 (v/v) mixture after dry ashing at 550 ˚C for 5 h. Leaf Ca content was determined using atomic absorption spectroscopy (Shiri *et al.,* 2016).

Data were statistically analyzed in SAS 9.1, and Duncan's multiple range test at 5% probability was used to compare the mean values.

RESULTS

Flower length

The results indicated that gladiolus flower length was significantly affected by the foliar application of Ca in both years (Tables 1 and 2). Flower length was increased as the Ca doses were increased (Table 1). In the 1st season, the tallest flower length (90.5 cm) was produced by the plants treated with 1.2 g L^{-1} Ca (Table 1). In the 2nd season, the highest flower length (109.6 cm) was obtained from the plots treated with 1.5 g L^{-1} Ca (Table 2). Flower length has shown significant regression with Ca doses ($P<0.001$, $y = -0.1539x^2 + 2.5133x + 80.932$, $y = -0.3115x^2 + 4.7141x + 92.423$ in the 1st and 2nd year, respectively). Ca source had a significant effect on spike elongation in both seasons (Tables 1 and 2). The data showed that calcium nitrate produced longer gladiolus length than calcium chloride (89.1 and 106.1 cm, respectively) in both years (Tables 1 and 2).

Table 1.This means comparison and variance analysis of the effect of different calcium sources and doses on the growth and quality of *Gladiolus* (1st year).

Ca Concentration/ type	Flower length (cm)	Spike length (cm)	Stem diameter (cm)	Flower fresh weight (g)	Flower dry weight (g)	Bending$_1$ (cm)	Bending$_2$ (cm)	Vase life (day)	Ca (%)
Ca_0	83.63 ^{b}	30.35^{b}	0.97^{b}	179.27^{b}	51.05^{c}	13.92^{a}	17.57^{a}	16.73^{c}	0.608^{c}
$Ca_{0.3}$	85.10 ^{ab}	31.85^{ab}	1.02^{ab}	188.68^{ab}	56.07^{bc}	13^{ab}	16.55^{a}	17.85^{bc}	0.673^{bc}
$Ca_{0.6}$	86.30 ^{ab}	33.17^{ab}	1.06^{ab}	201.1^{ab}	62.39^{ab}	12.25^{ab}	15.45^{ab}	18.57^{bc}	0.688^{bc}
$Ca_{0.9}$	88.92 ^{ab}	34.40^{ab}	1.13^{a}	211.75^{ab}	65.98^{ab}	11.42^{ab}	14.80^{abc}	19.57^{ab}	0.715^{ab}
$Ca_{1.2}$	90.48 ^{a}	35.30^{a}	1.14^{a}	220.25^{a}	69.84^{a}	10.87^{b}	13.32^{bc}	21.17^{a}	0.763^{ab}
$Ca_{1.5}$	89.93 ^{a}	34.98^{ab}	1.1^{a}	216.2^{ab}	70.64^{a}	10.08^{b}	11.88^{c}	21^{a}	0.785^{a}
$CaNO_3$	89.10^{a}	34.54^{a}	1.11^{a}	207.56^{a}	64.8^{a}	10.83^{a}	14.18^{b}	20.21^{a}	0.729^{a}
$CaC1_2$	85.16^{b}	32.11^{ab}	1.04^{b}	198.13^{b}	60.52^{ab}	13.02^{b}	15.68^{a}	18.19^{b}	0.682^{b}

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

S.o.V	df	MS								
Replication	2	24.55	0.30	0.003	6.89	139.72	0.90	0.70	1.27	0.005
Ca doses	5	46.70**	22.17**	0.03**	1578.3**	365.48*	11.10**	26.1**	18.64**	0.025**
Ca Sources	1	104.72**	53.05**	0.05**	799.0*	164.87^{ns}	43.12**	20.3**	40.22**	0.021*
Ca doses × Ca Sources	5	1.67^{ns}	1.62^{ns}	0.002^{ns}	48.89^{ns}	5.8^{ns}	0.39^{ns}	1.23^{ns}	1.02^{ns}	0.001^{ns}
Error	22	10.91	1.3	0.006	185.01	92.87	2.83	1.02	1.73	0.003
CV (%)		3.87	3.42	7.18	6.71	15.38	14.10	6.77	6.87	8.19

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$, and insignificant based on Duncan's test, respectively.

Table 2.Variance analysis (ANOVA) for the effects of calcium sources and doses on the growth and quality of *Gladiolus* (2nd year).

Ca Concentration/ type	Flower length (cm)	Spike length (cm)	Stem diameter (cm)	Flower fresh weight (g)	Flower dry weight (g)	Bending$_1$ (cm)	Bending$_2$ (cm)	Vase life (day)	Ca (%)
Ca_0	96.6^{d}	35.67^{b}	1.007^{b}	117.99^{b}	30.93^{b}	16.06^{a}	18.6^{a}	9.33^{b}	0.668^{d}
$Ca_{0.3}$	101.13^{c}	36.99^{ab}	1.16^{ab}	125.07^{ab}	32.63^{ab}	15.28^{a}	17.89^{a}	10.83^{ab}	0.690^{cd}
$Ca_{0.6}$	103.44^{c}	38.13^{ab}	1.23^{ab}	135.33^{ab}	34.62^{ab}	14.39^{ab}	16.11^{b}	11.5^{a}	0.710^{bc}
$Ca_{0.9}$	106.38^{b}	39.13^{a}	1.33^{a}	139.8^{ab}	36.48^{ab}	13.58^{ab}	15.5^{b}	12.33^{a}	0.737^{b}
$Ca_{1.2}$	108.03^{ab}	40.03^{a}	1.42^{a}	148.73^{a}	38.55^{a}	12.92^{ab}	14.62^{b}	12.75^{a}	0.777^{a}
$Ca_{1.5}$	109.6^{a}	40.54^{a}	1.43^{a}	149.33^{a}	37.98^{a}	11.26^{b}	14.55^{b}	12.7^{a}	0.787^{a}
$CaNO_3$	106.11^{a}	39.24^{a}	1.32^{a}	138.99^{a}	36.91^{a}	12.71^{b}	15.16^{b}	12.32^{a}	0.754^{a}
$CaC1_2$	102.28^{b}	37.57^{ab}	1.21^{ab}	133.09^{b}	33.49^{b}	15.12^{a}	17.32^{a}	10.83^{b}	0.702^{b}

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

S.o.V	df	MS								
Replication	2	94.1	11.11	0.027	18.51	37.28	9.46	3.05	1.4	0.001
Ca doses	5	139.69**	20.74*	0.16**	958.31**	54.89*	17.79**	82.72**	10.56**	0.013**
Ca Sources	1	131.91**	24.9^{ns}	0.1^{Ns}	314.24*	104.69*	52.08**	41.95**	19.8**	0.024**
Ca doses× Ca Sources	5	2.58^{ns}	1.53^{ns}	0.002^{ns}	3.63^{ns}	0.29^{ns}	0.57^{ns}	3.2^{ns}	0.59^{ns}	0.001^{ns}
Error	22	4.71	7.73	0.03	70.86	20.21	3.98	1.99	1.46	0.001
CV (%)		2.08	7.24	13.73	6.19	12.77	14.34	8.69	10.34	3.86

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01, and insignificant based on Duncan's test, respectively.

Spike length

The data shown in tables 1 and 2 revealed that spike length was highly significantly affected by different Ca concentrations in both years. In general, higher Ca concentrations produced taller spikes. In the first year, Ca foliar application at 1.2 g L^{-1} resulted in the maximum spike length (35.2 cm) compared to the control (30.3 cm), and in the second year, it was 40 and 35.7 cm for the corresponding treatments, respectively. Spike length showed significant regression with Ca rates ($P<0.001$, $y = -0.1902x^2 + 2.3155x + 28.105$, $y = -0.0898x^2 + 1.6135x + 34.127$ in the 1st and 2nd years, respectively). Flowers that received calcium nitrate produced loner cut spikes (34.5 and 39.2 cm in the 1st and 2nd seasons, respectively) than those treated with calcium chloride (Tables 1 and 2).

Stem diameter

The data in tables 1 and 2 show that the Ca levels had a significant effect on gladiolus stem diameter. Increasing the Ca levels up to 1.2 g L^{-1} significantly increased stem diameter from 0.97 to 1.14 cm (Table 1) in the first year. In the second year, the stem diameter was 1.0 and 1.42 cm for the corresponding treatments, respectively (Table 2). Stem diameter showed significant regression with the Ca doses ($P<0.01$, $y = -0.011x^2 + 0.1087x + 0.8592$, $y= -0.0112x^2 + 0.1649x + 0.8577$ in the 1st and 2nd years, respectively). The results showed that flowers nourished with calcium nitrate had larger stem diameters (1.11 and 1.32 cm, respectively) than those treated with calcium chloride (1.04 and 1.21 cm, respectively) in both years.

Flowering stem fresh weight

Flower fresh weight was affected significantly by calcium foliar application in both years (Tables 1 and 2). In the first year, calcium foliar application at the rate of 1.2 g L^{-1} resulted in 220.3 g/spike flower fresh weight (Table 1). In the second year, the maximum flower fresh weights of 149.3 and 148.7 g/spike were observed at the Ca levels of 1.5 and 1.2 g L^{-1}, respectively with insignificant differences (Table 2). Flower fresh weight showed significant regression with calcium doses ($P<0.001$, $y= -1.4985x^2 + 18.75x + 159.95$, $y= -0.6741x^2 + 11.352x + 106.53$ in the 1st and 2nd years, respectively). In this study, calcium sources significantly influenced flower fresh weight in both years (Tables 1 and 2). In both years, the plants sprayed with calcium nitrate and calcium chloride exhibited the maximum fresh weight of 138.9 and 207.6 g/spike, respectively (Tables 1 and 2).

Flowering stem dry weight

The foliar application of Ca significantly affected flower dry weight in both years (Tables 1 and 2). In the first year, the foliar application of Ca at the rate of 1.5 g L^{-1} resulted in 70.64 g/spike flower dry weight with an insignificant difference from the treatment of 1.2 g L^{-1} Ca (69.8 g/spike, Table 1). In the second year, the highest dry weight of 38.6 g/spike was produced when the plants were sprayed with 1.2 g L^{-1} Ca, which did not significantly differ from the treatment of 1.5 g L^{-1} Ca (38 g/spike). Flower dry weight exhibited significant regression with the Ca doses ($P<0.05$, $y= -0.1965x^2 + 2.9427x + 27.881$, $y= -0.553x^2 + 7.9523x + 43.212$ in the 1st and 2nd years, respectively). The Ca sources had a significant effect on flower fresh weight in both years (Tables 1 and 2). Flowers treated with calcium nitrate produced higher dry weight (64.8 g/spike) than those treated with calcium chloride (36.9 g/spike).

Inflorescence bending

Gladiolus flower stem firmness was significantly affected by the Ca treatments at harvest time and 7 days later in both years (Tables 1 and 2). In the first year, the lowest stem bending (10.1 and 11.9 cm at harvest time and 7 days later, respectively) was obtained from the plants sprayed with 1.5 g L^{-1} Ca, which had an insignificant difference from the plants sprayed with 1.2 g L^{-1} Ca (Table 1). The maximum stem bending was observed in the control (with 13.9 and 17.6 cm for harvest time and 7 days later, respectively), significantly differing from the treatments of 1.2 and 1.5 g L^{-1} Ca in the first year (Table 1).

The lowest stem bending (11.3 and 14.6 cm for harvest time and 7 days after harvest, respectively) was obtained from the plants sprayed with 1.5 g L^{-1} Ca, which showed a significant difference from the control in the 2nd year (Table 2). The results showed that maximum stem bending was 16.1 and 18.6 cm for harvest time and 7 days later in control, respectively which was significantly different from the treatments of 1.2 and 1.5 g L^{-1} Ca in the 2nd year (Table 2). $Bending_1$ showed significant regression with the Ca doses ($P<0.001$, $y= 0.0262x^2 - 0.9376x + 14.807$, $y= -0.0627x^2 - 0.4712x + 16.513$ in the 1st and 2nd years, respectively). The same holds for $bending_2$ ($P<0.001$, $y= -0.0646x^2 - 0.6555x + 18.202$, $y= 0.1188x^2 - 1.6925x + 20.36$ in the 1st and 2nd years, respectively).

Calcium sources had a significant effect on the flower stem firmness in both years (Tables 1 and 2). The results showed that flowers nourished with calcium nitrate had less stem bending (10.1 and 11.9 cm for harvest time and 7 days after harvest, respectively) than those treated with calcium chloride in the first year. The corresponding values were 12.7 and 13.2 cm for the second year, respectively (Tables 1 and 2).

Vase life

Calcium concentrations had a significant effect on gladiolus vase life in both years (Tables 1 and 2). The means comparison indicated that the highest vase life (21.1 days) was related to the plants treated with 1.2 g L^{-1} Ca, differing from the control significantly (16.7 days) in the first year (Table 1). Further, the maximum vase life (12.8 days) was observed in plants treated with 1.2 g L^{-1} Ca, which differed significantly from the control (9.3 days) in the second year (Table 2). Vase life showed significant regression with the Ca doses ($P<0.001$, $y= -0.0515x^2 + 1.2828x + 15.438$, $P<0.05$, $y= -0.1563x^2 + 1.7628x + 7.775$ in the 1st and 2nd years, respectively). The results showed that calcium sources had a significant effect on gladiolus vase life in both years (Tables 1 and 2). The data revealed that the maximum vase life (20.2 and 12.3 days in the 1st and 2nd years, respectively) was obtained from the plants treated with calcium nitrate (Tables 1 and 2).

Leaf calcium content

A significant effect on leaf Ca content was observed as a result of the Ca foliar application in both years (Tables 1 and 2). The maximum leaf Ca content (0.78%) was obtained from the flowers sprayed with 1.5 g L^{-1} Ca, significantly differing from the control (0.61%) in the first year (Table 1). In the second year, the highest Ca content of the leaves (0.79%) was obtained from those sprayed with 1.5 g L^{-1} Ca, which displayed a significant difference from the control (Table 2). The leaf Ca content showed significant regression with the Ca doses ($P<0.001$, $y = -0.0015x^2 + 0.0441x + 0.5737$, $y= 0.0004x^2 + 0.0224x + 0.6438$ in the 1st and 2nd years, respectively).

The results showed that the maximum leaf Ca content (0.73 and 0.75% in the first and second years, respectively) was obtained from the flowers treated with calcium nitrate (Tables 1 and 2).

DISCUSSION

To gain marketable flowers with long and strong stems and fresh flowers, it is worthy to consider vegetative parameters in addition to flower buds during their cultivation. The loss of quality in plants' vegetative parameters would result in the loss of the commercial and economic value of the resulting cut flowers (Sharifi and Naderi, 2019). Calcium is a divalent cation that is extremely important in maintaining stem bending. This mineral also regulates the absorption of nutrients across plasma cell membranes. Calcium functions in plant cell elongation and division, cell membrane structure and permeability, nitrogen metabolism, and carbohydrate translocation (Khalaj and Noroozisharaf, 2020). It is known as the critical mineral nutrient for flower and fruit quality. There are several reasons for the high susceptibility of flowers and fruits to Ca deficiency (Marschner, 2012; Hosseini Farahi and Aboutalebi Jahroomi, 2018). Calcium absorbed from the soil solution is transported through the xylem stream chiefly to the leaves due to their high transpiration. Compared to fruits and flowers, leaves have much bigger surface area-to-volume ratios and many stomata densities. Pre-harvest foliar spray of calcium can directly increase calcium concentration in plants and can improve flower quality and quantity (Marschner, 2012; Mohammadbagheri and Naderi, 2017; Khalaj and Noroozisharaf, 2020). Our results revealed that the foliar application of different calcium sources and concentrations significantly influenced the vegetative growth, flowering, and longevity of gladiolus spikes.

The length of gladiolus stems and spikes were significantly enhanced by the Ca foliar application (sources and concentrations) compared to the control in both years. By increasing

the Ca rate, the length of inflorescence stems and spikes were increased compared to the control (Tables 1 and 2).

The main reason for increasing the length of gladiolus inflorescence stems and spikes might be the important functions of Ca in plant cell elongation (Khalaj and Noroozisharaf, 2020; Marschner, 2012; Seyedi *et al.,* 2013). These functions are clearly reflected in the positive significant correlation observed between Ca concentration and inflorescence stem length (R^2= 0.6 and R^2= 0.78, respectively) and spike length (R^2= 0.67 and R^2= 0.80, respectively) in both years (Tables 3 and 4). This finding is in accordance with the results of Ahmad and Rab's (2020) study on gladiolus and Zhao *et al*.'s (2019) study on peony.

Also, increasing the rate of calcium nitrate foliar application increased gladiolus stem diameter (Tables 1 and 2), which might mainly be attributed to an increase in the number of thickened cell layers and the sclerenchyma cell walls (Zhao *et al.,* 2019). The role of Ca in plant cell elongation and division has already been demonstrated (Marschner, 2012), distinctly reflected in the positive significant correlation (R^2= 0.51 and R^2= 0.66, respectively) observed between calcium concentration and inflorescence stem diameter in both years (Tables 3 and 4). Similar results have been reported by Choi *et al*. (2004) about lily flowers, Combrink (2018) about gerbera flowers, Mortazavi *et al*. (2016) about tuberose, and Zhao *et al*. (2019) about peony. Increasing the rate of Ca foliar application significantly enhanced gladiolus stem fresh and dry weight versus the control (Tables 1 and 2).

Foliar application by 1.5 g L^{-1} Ca increased the fresh and dry weight by 26.5 and 20.5% versus the control in the 1st year, respectively. The corresponding values were 38.4 and 22.8% versus the control in the 2nd year (Tables 1 and 2). Furthermore, the foliar application of calcium nitrate increased the fresh weight by 4.4 and 4.8% and the dry weight by 7.1 and 10.2 % in the 1st and 2nd year, respectively compared to calcium chloride (Tables1 and 2). The enhancing effect of the Ca foliar application can be explained based on its role in cell membrane structure as well as increasing photosynthetic and other metabolic activities related to cell division and elongation (Marschner, 2012; Sharma *et al.,* 2013). This role of Ca is distinctly reflected in the positive significant correlation between the Ca concentration and inflorescence fresh weight (R^2= 0.58 and R^2= 0.75 in the 1st and 2nd years, respectively) and dry weight (R^2=0.34 and R^2= 0.61 in the 1st and 2nd years, respectively) as shown in Tables 3 and 4. These findings are in line with those of Mortazavi *et al*. (2016) about tuberose and Zhao *et al*. (2019) about peony.

Gladiolus flowering stem firmness was significantly affected by calcium treatment at the harvest time and 7 days later in both years (Tables 1 and 2) and the least bending was obtained from the maximum foliar application concentration (1.5 g L^{-1} Ca) in both years.

There are two distinct areas in the cell wall with high Ca^{2+} concentrations, the middle lamella and the extension surface of the plasma membrane. In both sites, Ca^{2+} has fundamental structural functions, i.e., the regulation of membrane permeability and its related processes and the bending of cell walls (Hamedan *et al.,* 2019). Over 60% of the calcium in cells is found in their walls (Moallaye-Mazraei *et al*., 2020). Plant cell walls, especially the secondary wall, which act as the skeletal of plant frameworks make mechanical support to the entire plant body which is one of the physiological properties of calcium in plants (Hosseini Farahi and Aboutalebi Jahroomi, 2018; Ahmad and Rab, 2019). It has been proved that cell wall thickness in the sclerenchyma and the number of vascular bundles are the important factors that are responsible for the mechanical rigidity of stems and the delay of senescence processes (Marschner, 2012; Moallaye-Mazraei *et al*., 2020). A negative linear and highly-significant correlation (R^2=-0.45

and -0.68, respectively) was found for the harvest time and 7 days later between Ca content and stem bending in the first year as well as for the harvest time and 7 days later between Ca content and stem bending (R^2= -0.67 and -0.72, respectively) in the second year (Tables 3 and 4).

Table 3. Correlation of quantitative traits in *Gladiolus* (1st year).

Traits	Flower length	Spike length	Stem diameter	Flower fresh weight	Flower dry weight	Bending$_1$	Bending$_2$	Vase life	Ca
Flower length	1.00								
Spike length	0.63**	1.00							
Stem diameter	0.83**	0.66**	1.00						
Flower fresh weight	0.66**	0.74**	0.74**	1.00					
Flower dry weight	0.56**	0.68**	0.52**	0.70**	1.00				
Bending$_1$	-0.50**	-0.74**	-0.51**	-0.59**	-0.53**	1.00			
Bending$_2$	-0.52**	-0.74**	-0.45**	-0.69**	-0.52**	0.66**	1.00		
Vase life	0.64**	0.83**	0.66**	0.77**	0.69**	-0.59**	-0.67**	1.00	
Ca	0.60**	0.67**	0.51**	0.58**	0.34*	-0.45**	-0.69**	0.68**	1.00

*and **: Significant at P < 0.05 and P < 0.01 based on the Duncan test, respectively. bending$_1$: Flower stem bending at the harvest time and bending$_2$: Flower stem bending seven days after the harvest.

Table 4. Correlation of quantitative traits in *Gladiolus* (2nd year).

Traits	Flower length	Spike length	Stem diameter	Flower fresh weight	Flower dry weight	Bending$_1$	Bending$_2$	Vase life	Ca
Flower length	1.00								
Spike length	0.84**	1.00							
Stem diameter	0.71**	0.61**	1.00						
Flower fresh weight	0.82**	0.74**	0.63**	1.00					
Flower dry weight	0.63**	0.56**	0.45**	0.81**	1.00				
Bending$_1$	-0.65**	-0.56**	-0.58**	-0.46**	-0.34*	1.00			
Bending$_2$	-0.83**	-0.72**	-0.68**	-0.59**	-0.50**	0.67**	1.00		
Vase life	0.69**	0.71**	0.59**	0.74**	0.57**	-0.53**	-0.58**	1.00	
Ca	0.78**	0.80**	0.66**	0.75**	0.61**	-0.67**	-0.72**	0.72**	1.00

*and **: Significant at P < 0.05 and P < 0.01 based on the Duncan test, respectively. bending$_1$: Flower stem bending at the harvest time and bending$_2$: Flower stem bending seven days after the harvest.

These results are in agreement with those of Abdolmaleki *et al*. (2015) about rose and Aghdam *et al*. (2019) about gerbera who observed better quality and quantity characteristics by increasing the Ca spraying dose. In herbaceous peony (*Paeonia lactiflora* Pall.), high Ca^{2+} concentration in the inflorescence stem's cell walls was related to the higher thickness of secondary cell walls, which was associated with its role in enhancing the biosynthesis of monolignols by induced calcium signal transduction (CAM/CML, CIPK, and CDPK). This signaling is turned on by the activation of downstream Ca-binding transcription factors belonging to NAC and MYB families. NAC and MYB members are the main factors involved in upregulating secondary cell wall biosynthesis and the expressions of monolignol biosynthetic genes (PAL, C_4H, 4CL, CCR, CAD, CSE, COMT, and CCoAOMT).This leads to increased lignin production in the secondary cell wall components (Zhao *et al.,* 2019).

Cut spikes' vase life depends on different physical observations, such as the fall down of spike heads, the shedding of petals, and the discoloration of flowers. Vase life was evaluated as the time period during which the fresh weight of the spikes was retained at the same level as that on the initial day of harvest (Ezhilmathi *et al.,* 2007). Cut flower quality and increased vase life ensure customer satisfaction, which will guarantee the sustainability of the market producer (Zhao *et al.,* 2016). Therefore, any treatment, which enhances the flowering duration of the plant, will always be accepted universally. The data in Tables 1 and 2 indicated that increasing the calcium rate was the most effective in extending the spikes' longevity. An enhanced life cycle of cut gladiolus flowers was reported by Reddy and Sarkar (2016) with the use of calcium nitrate. The efficiency of calcium in increasing vase life was reported by Buchanan and Gruissem (2015) in roses and Ahmad and Rab (2020) in gladiolus. These effects of calcium ions on higher membrane integrity help maintain or enhance cell turgor pressure, which contributes to weight loss during postharvest storage (Khalaj *et al.,* 2017; Ahmad and Rab, 2019). Calcium has a fundamental role in membrane integrity and wall bending. Calcium binding to cellular membranes, particularly negatively charged phospholipids of cellular membranes, is essential to maintain the integrity and control its function (Aghdam *et al.,* 2019). Phospholipase D (PLD) activity is regulated by cytosolic Ca^{2+} concentration. An increase in cytosolic Ca^{2+} concentration in response to environmental stress and hormonal messages, such as ethylene, activates PLD (Aghdam *et al.,* 2019).

Calcium rate and sources had a significant effect on gladiolus vase life in both years (Table 1). This role of Ca is clearly observed in the positive significant correlation (R^2= 0.68 and R^2= 0.72 in the 1st and 2nd years, respectively) between calcium concentration and gladiolus vase life (Tables 3 and 4). Also, the results demonstrated that vase life had significant correlations with stem diameter (R^2= 0.66 and R^2= 0.59 in the 1st and 2nd years, respectively), flower fresh weight (R^2= 0.77 and R^2= 0.74 in the 1st and 2nd years, respectively), flower dry weight (R^2= 0.69 and R^2= 0.57 in the 1st and 2nd years, respectively) and stem bending (R^2= 0.59 and R^2= 0.53 in the 1st and 2nd years, respectively) as shown in Tables 3 and 4. As well, all of them had significant correlations with the plant calcium content. Similar results concerning the longevity of cut flowers have been presented by Khalaj *et al*. (2017) and Aghdam *et al.* (2019) about gerbera and Sharma *et al*. (2013) about gladiolus.

Calcium is an essential nutrient affecting plant growth processes such that its accumulation accelerates creating the relationship among pectin polymers, and consequently, it increases the mechanical bending of stem and lignin creation. Finally, they reduce stem bending and extend flower vase life (Khalaj *et al.,* 2017; Aghdam *et al.,* 2019).The present research revealed that calcium content in the gladiolus leaves increased by 29% and 17% in the 1st and 2nd years, respectively by increasing calcium foliar application from 0 to 1.5 g L^{-1} (Tables1 and 2). Moreover, the maximum leaf Ca content (0.73% and 0.75% in the 1st and 2nd years, respectively) was obtained from the flowers that grew under a calcium nitrate source (Tables 1 and 2). These results agreed with the findings of Aghdam *et al*. (2019) about gerbera and Ahmad and Rab (2020) about gladiolus. Pre-harvest calcium foliar application can directly increase calcium concentration in plants and can improve flower quality and quantity (Khalaj and Noroozisharaf, 2020; Marschner, 2012).

CONCLUSIONS

On the basis of aforesaid evaluation, we propose a pathway Ca program to enhance the quality and quantity parameters of cut spike gladiolus. Taken together, the results demonstrated

that the foliar application of different calcium sources (chloride or nitrate) increased flowering parameters in gladiolus including vase life, flower length, flower stem diameter, and other growth parameters that are important for producers and flower markets. Both forms of calcium had a positive effect on gladiolus, but chiefly calcium nitrate had a better effect on growth parameters. The best gladiolus growth parameters were achieved by spraying the plants with 1.5 g L^{-1} Ca from the calcium nitrate source.

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