

Calcium Chelated with Amino Acids Improves Quality and Postharvest Life of Lisianthus (*Eustoma grandiflorum* cv. Cinderella Lime)

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Abstract. Lisianthus is one of the most important specialty cut flowers in the world. Various greenhouse conditions and inadequate evapotranspiration can disturb the transport of calcium and impair its uptake by plants. This study aimed to compare the effects of calcium amino acid chelates and calcium chloride (CaCl_2) on flower production, quality, and postharvest life of cut ‘Cinderella Lime’ lisianthus. Therefore, nutrient solutions containing calcium amino acid chelates (1%) were prepared using calcium and equal concentrations of lysine, threonine, or methionine. The control treatment was a solution without amino acids and calcium. Calcium concentrations of flowering stems were significantly higher in plants treated with calcium amino acid chelates than those treated with amino acids or the control treatment. Treatment with calcium methionine chelate led to significantly higher flower numbers compared with treatment with free amino acids and the control treatment. Moreover, calcium amino acid chelates effectively improved the fresh and dry mass of the flowering stems in comparison with the control plants. In summary, among all calcium sources, calcium lysine chelate could most effectively enhance the postharvest life of lisianthus cut flowers.

The genus *Eustoma* belongs to Gentianaceae family and includes two species, i.e., *E. exaltatum* L. and *E. grandiflorum* (Raf.) Shinn, commonly known as Eustoma, lisianthus, prairie gentian, Texas bluebell (Dole and Wilkins, 2004). Lisianthus is a moderately cold-tolerant biennial plant and is grown as an annual for commercial purposes (Roh and Lawson, 1988). Besides as a cut flower, lisianthus is also available as potted or bedding plants (Dole and Wilkins, 2004). Considering lisianthus as a major cut flower in many countries (Anderson, 2007), its greenhouse production, especially using soilless culture systems, is more feasible. Several factors, particularly plant nutrition, may be involved in successful production of this flower in soilless culture systems. Among various nutrients, calcium plays an essential role in the growth, productivity, and quality of greenhouse-grown cut flowers. Calcium is

absorbed as Ca^{2+} ions. It uses passive transport channels and has very low mobility in plants (Barker and Pilbeam, 2007; Fageria, 2009; Mills and Jones, 1996). After absorption from soil colloids, calcium moves toward the roots through diffusion or mass movement and is absorbed by unsubsized root tips (Barker and Pilbeam, 2007). Therefore, any damage to root tips, caused by nematodes, chemical changes, or tension, can reduce calcium absorption. Both apoplastic and symplastic pathways are involved in the transfer of calcium from the soil solution to the vascular system (Mills and Jones, 1996). Calcium easily enters the apoplast and interchangeably adheres to cell walls and outer surface of cytoplasm membrane (White and Broadley, 2003). Numerous factors, such as metabolism processes, can restrict calcium absorption by the cytoplasm. Moreover, the transport of calcium from one cell to another and within the phloem occurs very slowly. Calcium seems to be the only element, except for boron, which performs most of its activities outside the cytoplasm, i.e., inside the apoplast. Since calcium uptake by plant roots is directly related with transpiration rate, any factor that affects transpiration can also influence calcium

uptake (Marschner, 1995). Certain environmental changes, such as increased moisture inside the greenhouses, especially in winter, can alter plant transpiration rates and thus calcium uptake and transport. Calcium deficiency will lead to various physiological disorders such as the bending of the peduncle and breaking of the stem, particularly in gerbera. Such symptoms usually manifest following decreased transpiration from the aerial parts of the plant (Gerasopoulos and Chebli, 1999). Decreased transpiration rates over long periods of cloudy weather will cause calcium deficiency and its symptoms burnt leaf tips and margins will appear in lisianthus (Dole and Wilkins, 2004). Although such a disorder will be initially observed during budding, persistent deficiency will lead to the bending and drooping of the peduncle. Spraying different organic and inorganic sources of calcium, e.g., $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, are applied during winter or early spring production to prevent calcium deficiency (Dole and Wilkins, 2004). Recently, Ca-amino acid chelates have been synthesized and distributed to supply different metal nutrients such as zinc and iron (Ghasemi et al., 2012, 2013). Amino acids can form a relatively stable complex with calcium and thus enhance its availability. It has been shown metal-amino acid chelates can easily pass through root cell wall pores (Ghasemi et al., 2013). Infiltration of these chelates into plant tissues and their slow degradation will prevent the binding of calcium with the anionic region of plant cell membranes (Saftner et al., 2003). Therefore, this study was performed to investigate first how different sources of calcium affect lisianthus production and then to study the effects of synthetic calcium amino acid chelates and CaCl_2 on qualitative and quantitative features of lisianthus cut flowers.

Materials and Methods

Plant material. Lisianthus seedlings (‘Cinderella Lime’) were purchased from Moshkelgosha Greenhouse (Isfahan, Iran). In Feb. 2013, the seedlings were transferred to the greenhouse at the Department of Horticulture (Isfahan University of Technology, Isfahan, Iran). During the course of the study (5 months), the mean temperature in the greenhouse was maintained at 28 ± 4 °C in the day and 18 ± 4 °C at night. Moreover, the relative air humidity (measured by HYGRO TFA, Germany) was 50% to 70%, in a 16 ± 2 h light/ 8 ± 2 h dark photoperiod cycle. The mean light intensity [measured by an auto ranging light meter (CEM DT-1309 Professional Digital 40K FC Foot-Candle Meter 400 klx Light Luxmeter, China)] was 650–740 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (average during the whole day). To ensure uniform conditions, seedlings with four to five pairs of leaves were selected and separately planted in 4-L pots containing a 1:1 volume ratio mixture of peatmoss and perlite. Moreover, a basic nutrient solution (half strength modified Johnson’s nutrient solution) was prepared using distilled water (Table 1) and applied for 15–20 d to equalize plant

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growth. Afterward, the plants were pinched above the sixth node (to facilitate the treatments), calcium nitrate (i.e., the calcium source) was eliminated from the nutrient solution, and ammonium nitrate was added to compensate for the eliminated nitrogen (Table 2). According to Table 2, in calcium treatments, the amount of calcium required by the plants was provided through hydrous CaCl_2 and calcium amino acid chelates (2 mM). Ca amino acid chelates were prepared from Qom Zist Fanavar-e-Novin Co. 11831, Iran. The electrical conductivity (measured by Az Temp/conductivity meter 86503, Taiwan) and acidity (measured by pH Laboratory 827 Metrohm, Switzerland) of the nutrient solution were 1.8–2 and 5.7–6.5 dS/m, respectively. Three months after transferring the seedlings into the pots, the flowering stems with at least two fully blossomed buds were cut (left a node on the stem). They were then moved to a container filled with distilled water and qualitative and quantitative measurements were performed. Other characteristics including diameter and number of flowers, diameter, length, number and, dry and fresh weight of flower stems were also measured. The presented data are the average of nine pots.

Nutrient content. After separating the petals, the flowering stems and their leaves were dried at 70 °C for 48 h. The dried samples were ground and passed through a sieve (mesh size: 30 μm). The powders were then heated at 550 °C for 6 h and the resulting ash was collected. To obtain extracts, the crucibles were left to cool down and then 10 mL of 2 M hydrochloric acid (HCL) were added to the samples. The resultant solution was passed through a filter paper and the extract was diluted to 100 mL using distilled water. A Perkin-Elmer atomic absorption spectrophotometer (PerkinElmer 3030 Inc., Waltham, MA) was finally used to measure the calcium content of the flowering stems and their leaves (Volpin and Elad, 1991).

Vase life and relative fresh weight. To perform postharvest evaluations, the flowering stems were harvested early in the morning. All stems were diagonally trimmed (under water) to 57 ± 2 cm. Two open flowers and four flower buds were left on each stem and the remaining flowers and buds were removed (Shimizu-Yumoto and Ichimura, 2010). The stems were moved to the laboratory and kept in containers holding 500 mL distilled water. The containers were maintained at a temperature of 26 ± 2 °C, relative humidity of $60\% \pm 5\%$, and light/dark cycle of 16:8. The containers had been disinfected with a 0.1% sodium hypochlorite solution for 45 min. Vase life was defined as the period between the harvest and the time when the wrinkling and browning of the petals and bending of the peduncle started. Fresh weights of nine stems per treatment were recorded every second day for 14 d. The distilled water was also replaced every 2 d (Shimizu-Yumoto and Ichimura, 2010). The flower stem weights on the first and 14th days were finally subtracted and the reduction was reported in percentages.

Data analysis. The experiment composed three replicates of randomized complete blocks. Since each treatment consisted of five pots (each containing one plant), a total of 120 pots were prepared. The generalized linear model analysis of variance was conducted to assess the effects of different treatments. The mean values were also compared using the least significant difference (LSD). All analyses were performed with Statistical Analysis Software 2000 (SAS Institute, Cary, NC) at a significance level of $P < 0.05$.

Results

Fresh and dry weight, number and length of the stems. Compared with the control treatment, the application of calcium amino acid chelates significantly increased fresh and dry mass of the stems. In contrast, such

an effect was absent when free amino acids and CaCl_2 were applied. Fresh and dry weight of the flowering stems were 29.82% and 20.35% higher in calcium lysine-treated plants than in the control treatment ($P < 0.05$) (Table 3). In fact, among all calcium amino acid chelates and free amino acids, calcium lysine and free lysine had the greatest effects on increasing fresh and dry weight of the flowering stems. Addition of calcium, regardless of the source, was ineffective on stem number and length (Table 3).

Diameter of the flowers and stems. As shown in Fig. 1., plants treated with free methionine had significantly lower flower diameter compared with those receiving other amino acids and calcium amino acid chelates. Addition of calcium amino acid chelates had no significant effects on flower diameter. Moreover, CaCl_2 had no significant effects on flower diameter. A significant difference in

Table 1. A basic nutrient solution (half strength modified Johnson's nutrient solution without calcium).²

Macro elements	Source	Concn in solution (meq/L)
N	KNO_3 , $\text{NH}_4\text{H}_2\text{PO}_4$, NH_4NO_3	8
K	KNO_3	3
Mg	MgSO_4	1
P	$\text{NH}_4\text{H}_2\text{PO}_4$	2
S	$\text{MgSO}_4 \cdot 0.7\text{H}_2\text{O}$, MnSO_4 , $\text{ZnSO}_4 \cdot 0.7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	1
Micro elements		μM
Fe	Fe-EDTA	50
Mn	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	2
Zn	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	2
B	H_3BO_3	25
Cu	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.5
Mo	MoO_3	0.5

²To remove the calcium source, calcium nitrate was eliminated from the nutrient solution after 15–20 d of providing the potted plants with half strength modified Johnson's nutrient solution containing calcium. Ammonium nitrate was used to compensate for the eliminated nitrogen.

Table 2. Details of calcium treatments.

Number	Treatment	Name	Molecular formula	Molecular wt
1	Ca (Lys) ₂	Calcium-lysine chelate (1%)	—	186.19
2	Ca (Met) ₂	Calcium-methionine chelate (1%)	—	189.21
3	Ca (Thr) ₂	Calcium-threonine chelate (1%)	—	159.12
4	Aa Lys	Lysine amino acid (1%)	$\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2$	146.19
5	Aa Met	Methionine amino acid (1%)	$\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$	149.21
6	Aa Thr	(1%) Threonine amino acid	$\text{C}_4\text{H}_9\text{NO}_3$	119.12
7	CaCl_2	Calcium chloride (290 mg/L)	CaCl_2	147.02
8	Control	Control (half strength modified Johnson's nutrient solution without calcium salt)	—	—

Table 3. Fresh and dry weight, length and number of stems in lisianthus ('Cinderella Lime') plants as affected by different sources of calcium in the nutrient solution.

Treatment	Flower stems			
	Fresh wt (g/plant)	Dry wt (g/plant)	Length (cm)	Number
Ca (Lys) ₂	306.41 ± 3.83 a ²	139.85 ± 3.89 a	56.65 ± 1.76 a	3.93 ± 0.05 a
Ca (Met) ₂	287.96 ± 10.34 ab	134.56 ± 6.75 ab	53.96 ± 3.26 a	3.6 ± 0.11 a
Ca (Thr) ₂	268.07 ± 10.43 ab	129.67 ± 1.71 ab	54.65 ± 0.68 a	3.3 ± 0.11 a
Aa Lys	236.96 ± 19.11 bc	118.73 ± 5.28 bc	54.24 ± 1.95 a	3.46 ± 0.29 a
Aa Met	217.1 ± 24.47 c	111.74 ± 13.70 c	50.75 ± 1.66 a	3.11 ± 0.30 a
Aa Thr	206.94 ± 25.07 c	107.43 ± 13.97 c	53.91 ± 2.34 a	2.86 ± 0.35 a
CaCl_2	256.27 ± 29.20 abc	125 ± 13.22 abc	57.95 ± 2.73 a	3.26 ± 0.35 a
Control	215.02 ± 7.93 c	111.39 ± 3.03 c	53.37 ± 0.14 a	3.2 ± 0.11 a
LSD	52.54	20.28	5.93	0.96
CV	12.06	9.59	6.22	14.38

CV = coefficient of variation.

²Means with the same letter within the columns are not significantly different according to the least significance differences (LSD) test with $P \leq 0.05$. Average (measurements in nine plants per treatment) \pm SE.

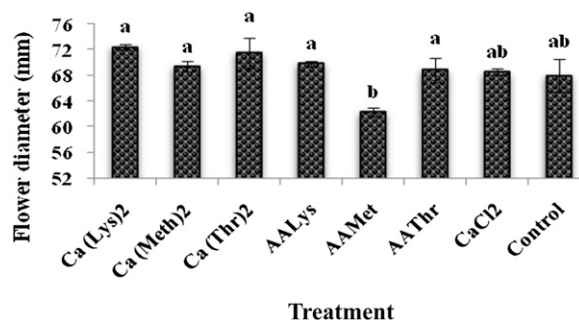


Fig. 1. The flower diameter of lisianthus ('Cinderella Lime') plants as affected by different sources of calcium in the nutrient solution. Error bars represent standard error (n = 3). Bars having different letters are significantly different at the 5% level by least significant difference (LSD).

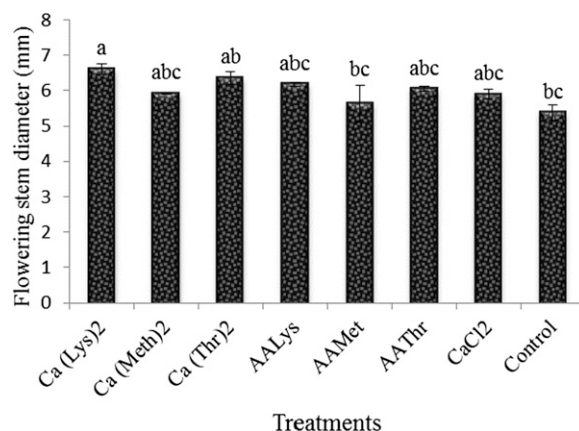


Fig. 2. The flowering stem diameter of lisianthus ('Cinderella Lime') plants as affected by different sources of calcium in the nutrient solution. Error bars represent standard error (n = 3). Bars having different letters are significantly different at the 5% level by least significant difference (LSD).

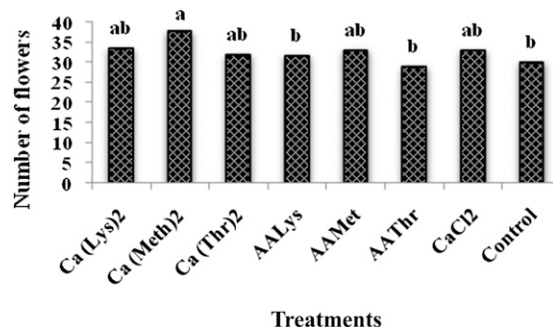


Fig. 3. The number of flowers of lisianthus ('Cinderella Lime') plants as affected by different sources of calcium in the nutrient solution. Error bars represent standard error (n = 3). Bars having different letters are significantly different at the 5% level by least significant difference (LSD).

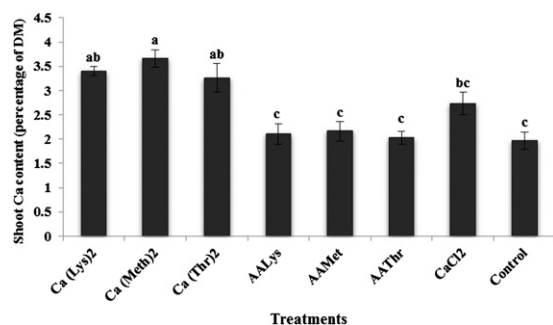


Fig. 4. The stem calcium content of lisianthus ('Cinderella Lime') plants as affected by different sources of calcium in the nutrient solution. Error bars represent standard error (n = 3). Bars having different letters are significantly different at the 1% level by least significant difference (LSD).

stem diameter was found between the calcium lysine and control treatments. Furthermore, calcium lysine improved stem diameter by 18.73% as compared with the control treatment (Fig. 2).

Flower number. The highest number of flowers was achieved by the addition of calcium methionine. Treatment with this compound resulted in significant differences with the control plants and free lysine- and threonine-treated plants. Calcium methionine increased flower number by 20.22% compared with the control treatment (Fig. 3).

Stem calcium content. The application of calcium amino acid chelates significantly increased calcium content of the stems compared with the control treatment. However, CaCl₂ and free amino acids had no significant effects on stem calcium content. Shoot calcium concentration was 25.40% and 46.44% higher in plants received calcium methionine amino chelates than in the plants treated with CaCl₂ and the control treatments, respectively (Fig. 4).

Vase life. Calcium amino acid chelates significantly enhanced vase life of lisianthus cut flowers in comparison with CaCl₂. In fact, calcium lysine improved vase life by 38.21% compared with the control treatment (Table 4).

Relative fresh weight. Over the 14-d post-harvest period, minimum percentage reduction in stem fresh weight was seen in plants treated with calcium amino acid chelates. However, there were no significant differences in weight reduction between the control treatment and plants treated with free amino acids (Fig. 5).

Discussion

Lysine, methionine, and threonine, which are all essential to animal and human health, are used in the synthesis of calcium amino acid chelates (Dawson, 1959). According to our findings, calcium amino acid chelates could also substantially increase shoot fresh and dry weight (Table 3). Similarly, Albino-Garduno et al. (2008) reported high calcium concentrations to increase dry stem weight in gerbera (Amaretto and Darling varieties). Raising calcium concentration of nutrient solutions has also been suggested to elevate calcium levels in plant tissues and increase stem dry weight in lisianthus cut flowers (Frett et al., 1988). In other words, higher calcium concentration in the nutrient solution will be associated with greater stem fresh and dry weight (Picchioni et al., 2001). The improved shoot fresh and dry weight in this study can also be justified by the presence of calcium and amino acids, which are believed to promote calcium absorption (through chelate formation) and enhance yield, mRNA transcription, synthesis of glucose, and protein content in plants (Ghasemi et al., 2012; Keutgen and Pawelzik, 2008; Rashad et al., 2003). However, we could not establish any significant differences in shoot number and length between various groups. In contrast, Shams et al.

(2012) reported treatment with high levels of calcium (7.5 mM) to maximize the length of the flowering stems in roses (Shams et al., 2012). Some researchers have also introduced the hormone-like activity and nitrogen content of amino acids to be responsible for growth promotion in plants (Madrid et al., 2003; Yeoh and Troung, 1996).

Nevertheless, providing higher amounts of calcium in nutrient solution does not always guarantee higher calcium content in the flower stem. For instance Bar-Tal et al. (2001) reported different levels of calcium not to have a significant effect on flower production in Mercedes, First Red, and Escada roses (Bar-Tal et al., 2001). Similar results were also indicated in a study on alstroemeria (Smith et al., 1998). On the contrary, Shams et al. (2012) indicated higher number of stems following the application of higher levels of calcium in cut roses (Shams et al., 2012). Although some researchers have attributed the increased number of stems to higher levels of calcium supply, Smith et al. (1998) and Bar-Tal et al. (2001) asserted the absence of a significant relation between calcium levels and number of stems. In our study, while the mean number of stems was higher in plants treated with calcium amino acid chelates, the difference between the groups was not

significant (Table 3). Postharvest quantitative assessments in this study revealed the significant positive effects of calcium-containing treatments on flower and stem diameter, flower number, vase life, and percentage reduction in fresh weight of the harvested cut flowers. Calcium amino acid chelates (particularly calcium lysine chelate) were the most effective on qualitative features, especially postharvest life of the flowers. Previous research has also confirmed the direct relation between higher calcium concentrations in nutrient solution and enhanced flower and stem diameter consequent to increased calcium content in plants. High calcium concentration (7.5 mM) in the nutrient solution has been reported to increase the diameter of the flowering stem in roses (Shams et al., 2012). It can in fact be concluded that the application of calcium amino acid (particularly lysine) chelates can promote the absorption of calcium by the flowering stem and hence increase flower and shoot diameter in comparison with control plants. Nikbakht et al. (2008) also tried to improve calcium uptake of hydroponically grown gerbera plants using humic acid (a naturally occurring chelating agent). According to their findings, any method that elevated calcium content in flower scapes could improve postharvest life of the flowers (Nikbakht et al., 2008). Frett et al. (1988) established a relation between increasing calcium content in the nutrient solution and augmented number of flower buds in lisianthus cut flowers (Frett et al., 1988). In a comparison between low and high calcium concentrations, Albino-Garduno et al. (2008) found the latter to increase the number of flowers in Amaretto and Darling varieties of gerbera (Albino-Garduno et al., 2008). Moreover, the absence of calcium was followed by reduced number of flowers in petunias (Frett et al., 1985). Calcium amino acid chelates could significantly enhance the calcium content of lisianthus (Fig. 4). Therefore, calcium treatments, especially calcium amino acid chelates, in this research seem to have facilitated calcium absorption by plant tissues and hence amplified the number of flowers. Based on previous studies, boosting calcium content in plant tissues would reduce ethylene production in petals, delay

senescence, and ultimately improve quality and vase life in many cut flowers including roses (Bar et al., 2009; Nabigol, 2012; Starkey and Pedersen, 1997; Torre et al., 1999; Volpin and Elad, 1991). In a study on recently harvested fresh honeydew, Saftner et al. (2003) reported calcium content of the tissues to be twice higher in samples dipped in a combination of sodium hypochlorite, calcium propionate, calcium amino acid chelate, and calcium salt than in control samples and tissues treated with sodium hypochlorite alone. The mentioned combination could also prevent changes in firmness, surface color, and tissue translucency during storage. Among all calcium treatments, calcium amino acid chelates were the most effective (Saftner et al., 2003). These findings about the positive effects of calcium on delayed senescence and postharvest life were consistent with the results of this study. We also detected greater enhancement in vase life in lisianthus cut flowers treated with calcium amino acid chelates than in control plants and those treated with amino acids or CaCl_2 . Similarly, nutrient solutions with higher calcium levels were found to increase calcium content and elevate fresh weight and delay its reduction in fresh Mercedes and Baroness roses (Torre et al., 1999) as well as Cool Water and Pretty Blinda roses (Nabigol, 2012). In other words, increased calcium content of the flowering stem will maintain the initial fresh weight and postpone weight reduction during postharvest stages (Nikbakht et al., 2008; Saftner et al., 2003; Torre et al., 1999). According to our findings, treatment with calcium-containing compounds, especially calcium amino acid chelates, improved initial fresh weight and limited weight reduction. These beneficial effects were more considerable than those exerted by amino acids and the control treatment. Comparison of all treatments in this research showed that the application of calcium amino acid chelates improved calcium content in flowering stems. Moreover, calcium content of stems treated with calcium methionine chelate was significantly higher than that in CaCl_2 -treated plants. Similarly, Saftner et al. (2003) found the greatest level of calcium in the tissues of harvested honeydew treated with calcium amino acid chelates (Saftner et al., 2003). The favorable effects of calcium amino acid chelates on calcium absorption can be justified by the presence of amino acids possessing hormone-like activity and capable of forming chelates with various elements (Madrid et al., 2003; Yeoh and Troung, 1996).

Conclusion

The results of this study confirmed the effectiveness of calcium amino acid chelates in increasing calcium absorption and shoot fresh and dry mass of lisianthus ('Cinderella Lime'). These effects were more favorable than those exerted by CaCl_2 . Since calcium amino acid chelates could preserve lisianthus cut flowers, they can be used as an appropriate source of calcium compared with CaCl_2 to improve the vase life of

Table 4. Vase life of lisianthus ('Cinderella Lime') plants as affected by different sources of calcium in the nutrient solution.

Treatment	Vase life (day)
Ca (Lys) ₂	16.72 ± 0.16 a ²
Ca (Met) ₂	14.77 ± 0.11 b
Ca (Thr) ₂	15.05 ± 0.14 b
Aa Lys	11.33 ± 0.19 d
Aa Met	10.05 ± 0.14 e
Aa Thr	10.33 ± 0.38 e
CaCl ₂	14.05 ± 0.14 c
Control	10.33 ± 0.19 e
LSD	0.53
CV	2.7

CV = coefficient of variation.

²Means with the same letter within the columns are not significantly different according to the least significance differences (LSD) test with $P \leq 0.05$. Average (measurements in nine plants per treatment) ± SE.

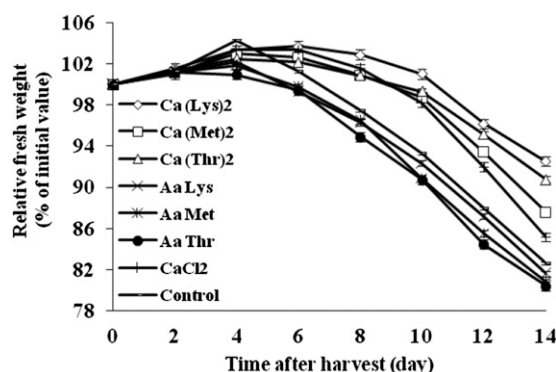


Fig. 5. The relative fresh weight of lisianthus ('Cinderella Lime') plants as affected by different sources of calcium in the nutrient solution at different times after harvest. Vertical bars represent the standard error (n = 8). Bars having different letters are significantly different at the 1% level by least significant difference (LSD).

lisianthus cut flowers. Simultaneous effects of calcium and amino acids were found to be responsible for the mentioned benefits of calcium amino acid chelates. According to the obtained results, calcium-lysine is recommended as a preferable source of calcium instead of CaCl_2 .

Literature Cited

- Albino-Garduno, R., H. Zavaleta-Mancera, L. Ruiz-Posadas, M. Sandoval-Villa, and A. Castillo-Morales. 2008. Response of gerbera to calcium in hydroponics. *J. Plant Nutr.* 31:91–101.
- Anderson, N.O. 2007. Flower breeding and genetics. Springer, Amsterdam, The Netherlands.
- Bar, B.Y., N.S. Mattson, and H.J. Lieth. 2009. Effect of $\text{NH}_4\text{:NO}_3$ ratio on cut roses yield, leaf nutrients content and proton efflux by roots in closed hydroponic system. *J. Sci. Hort.* 122:610–619.
- Barker, A.V. and D.J. Pilbeam. 2007. Handbook of plant nutrition. CRC Press, Boca Raton, FL.
- Bar-Tal, A., R. Baas, R.G. Neumann, A. Dik, N. Marissen, A. Silber, S. Davidov, A. Hazan, B. Kirshner, and Y. Elad. 2001. Rose flower production and quality as affected by Ca concentration in the petal. *J. Agron.* 21:393–402.
- Dole, S.E. and H.F. Wilkins. 2004. Floriculture principles and species. 2nd ed. Pearson Education, Upper Saddle River, NJ.
- Dawson, R.M.C. 1959. Data for biochemical research, Clarendon Press, Oxford, UK.
- Fageria, N.K. 2009. The use of nutrients in crop plants. CRC Press, Boca Raton, FL.
- Frett, J.J., M.A. Dirr, and A.M. Armitage. 1985. Nitrogen and calcium nutrition of *Petunia x hybrida* "Coral Sea". *J. Sci. Hort.* 26:351–359.
- Frett, J.J., J.W. Kelly, B.K. Harbaugh, and M. Roh. 1988. Optimizing nitrogen and calcium nutrition of lisianthus. *J. Commun. Soil Sci. Plant Anal.* 19:13–24.
- Gerasopoulos, D. and B. Chebli. 1999. Effect of pre- and postharvest calcium application on the vase life of cut gerbera. *J. Hort. Sci. Biotechnol.* 74:78–81.
- Ghasemi, S., A.H. Khoshgoftarmanesh, H. Hadadzadeh, and M. Afyuni. 2012. Synthesis of iron-amino acid chelates and evaluation of their efficacy as iron source and growth stimulator for tomato in nutrient solution culture. *J. Plant Growth Regul.* 31:498–508.
- Ghasemi, S., A.H. Khoshgoftarmanesh, M. Afyuni, and H. Hadadzadeh. 2013. The effectiveness of foliar applications of synthesized zinc-amino acid chelates in comparison with zinc sulfate to increase yield and grain nutritional quality of wheat. *J. Agron.* 45:68–74.
- Keutgen, A. and E. Pawelzik. 2008. Contribution of amino acids to strawberry fruit quality and their relevance as stress indicators under NaCl salinity. *J. Food Chem.* 111:642–647.
- Madrid, F., M.S. Liphadzi, and M.B. Kirkham. 2003. Heavy metal displacement in chelate irrigated soil during phytoremediation. *J. Hyrol.* 272:107–119.
- Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic press, London, UK.
- Mills, H.A. and J.B. Jones. 1996. Plant analysis handbook II. Micro-Macro Publishing, Athens, GA.
- Nabigol, A. 2012. Pre-harvest calcium sulfate application improves postharvest quality of cut rose flowers. *J. Biotechnol.* 11:1078–1083.
- Nikbakht, A., M. Kafi, M. Babalar, Y.P. Xia, A. Luo, and N. Etemadi. 2008. Effect of humic acid on plant growth, nutrient uptake and post-harvest life of gerbera. *J. Plant Nutr.* 31:2155–2167.
- Picchioni, G.A., M.V. Vazquez, and S.A. Sanchez. 2001. Calcium-activated root growth and mineral nutrient accumulation of *Lupinus hvardii*: Ecophysiological and horticultural significance. *J. Amer. Soc. Hort. Sci.* 126:631–637.
- Rashad, E.S.M., H.M. El-Abagg, and A.A. Amin. 2003. Physiological effects of some bioregulators on growth and productivity of two broad bean cultivars. *Egypt. J. Appl. Sci.* 18:132–149.
- Roh, S.M. and R.H. Lawson. 1988. Tissue culture in the improvement of *Eustoma*. *HortScience* 23:658 (abstr.).
- Saftner, R.A., J. Bai, J.A. Abbott, and Y.S. Lee. 2003. Sanitary dips with calcium propionate, calcium chloride, or a calcium amino acid chelate maintain quality and shelf stability of fresh-cut honeydew chunks. *J. Postharvest Biol. Technol.* 29:257–269.
- Shams, M., N. Etemadi, B. Baninasab, A.A. Ramin, and A.H. Khoshgoftarmanesh. 2012. Effect of boron and calcium on growth and quality of Easy Lover cut rose. *J. Plant Nutr.* 35:1303–1313.
- Shimizu-Yumoto, H. and K. Ichimura. 2010. Combination pulse treatment of 1-naphthaleneacetic acid and aminoethoxyvinylglycine greatly improves postharvest life in cut *Eustoma* flowers. *J. Postharvest Biol. Technol.* 56:104–107.
- Smith, M.A., G.C. Elliott, and M.P. Bridgen. 1998. Calcium and nitrogen fertilization of Alstroemeria for cut flower production. *J. Hort. Sci.* 33:55–59.
- Starkey, K.R. and A.R. Pedersen. 1997. Increased levels of calcium in the nutrient solution improves the postharvest life of potted roses. *J. Amer. Soc. Hort. Sci.* 122:863–868.
- Torre, S., A. Borochov, and A.H. Halevy. 1999. Calcium regulation of senescence in rose petal. *J. Physiol. Plantarum.* 107:214–219.
- Volpin, H. and Y. Elad. 1991. Influence of calcium nutrition on susceptibility of rose flowers to botrytis blight. *J. Physiol. Biochem.* 81:1390–1394.
- White, P.J. and M.R. Broadley. 2003. Calcium in plants. *J. Annals of Botany.* 92:487–511.
- Yeoh, H.H. and V.D. Troung. 1996. Protein contents, amino acid compositions and nitrogen-to-protein conversion factors for cassava roots. *J. Sci. Food Agr.* 70:51–54.