Calcium Ameliorates the Tolerance of Lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] to Alkalinity in Irrigation Water

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Abstract. Irrigation water high in alkalinity can severely compromise the growth and marketability of ornamental plants. In the present study we investigated the response of lisianthus to increased calcium (Ca) when irrigated with solutions containing high levels of bicarbonate (HCO_3^-)-induced alkalinity. Alkalinity in irrigation water reduced the growth of lisianthus; however, plants supplemented with an increased concentration of Ca at alkalinity levels from 4 to 7 meq·L⁻¹ of HCO_3^- exhibited improved growth and dry mass (DM) accumulation or were not detrimentally affected, demonstrating that Ca contributes to the increase of the tolerance of lisianthus to alkalinity. Supplementary Ca did maintain a high stomatal conductance (g_s) and transpiration rate when alkalinity was at 4 meq·L⁻¹, which explained the lower water potential in young leaves. Plants irrigated with solutions containing supplementary Ca had higher total DM, which was associated with a higher g_s ; however, when conductance was higher than 0.280 cm·s⁻¹, like in plants with no supplementary Ca, DM tended to decrease. At a typical Ca concentration, there was a disruption on stomata functioning as g_s and transpiration rate increased, which was associated with a reduction in shoot potassium (K). Calcium ameliorated the uptake of K when alkalinity was 4 meq·L⁻¹ by allowing a less marked reduction in shoot K concentration. Chlorophyll was reduced by increasing alkalinity as a result of a decrease in shoot iron (Fe); however, supplementary Ca also contributed in increasing plant tolerance to alkalinity at 4 meq·L⁻¹ by sustaining a high shoot Fe concentration. Supplementary Ca increased catalase and peroxidase activities, indicating that lisianthus responded to the stress by enhancing the activity of these enzymes to reduce oxidative damage.

Alkalinity affects plant production in the arable lands of the arid and semiarid regions of the world. Irrigation water of high alkalinity may cause nutritional problems in cultivated horticultural plants, specifically chlorosis in young leaves resulting from the formation of Fe forms that are unavailable for plant uptake. Alkalinity may also be associated with deficiency of copper (Cu), zinc (Zn), K, and phosphorus (P) resulting from low solubility (FAO, 2000).

Alkalinity in water is caused mainly by carbonate (CO_3^{2-}) and HCO_3^- , which at high concentrations are detrimental for plant growth (Cartmill et al., 2007). Use of irrigation water of high alkalinity leads to increased pH of the

growing medium because acidity is neutralized by HCO_3^- and CO_3^{2-} (Valdez-Aguilar and Reed, 2007).

High alkalinity irrigation water results in reduced growth and nutrient deficiency symptoms in young leaves, reducing plant quality and marketability, and represents a significant challenge to ornamental horticultural production systems. Lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.], a species native to the arid zones of the southern United States and northern México, is an ornamental plant of increasing demand. Valdez-Aguilar et al. (2013) reported that lisianthus can tolerate high salinity irrigation water as long as sodium and chloride are not the predominant ions.

Calcium has been reported in a number of studies as an ion that enhances plant tolerance to abiotic stress, including drought (Hassan and Aarts, 2011), salinity (Zhu, 2001), and excess boron (Siddiqui et al., 2013) by

regulating the response reactions and developmental processes (Steinhorst and Kudla, 2013). However, there is limited information on the potential role of Ca in enhancing plant tolerance to alkalinity stress. Calcium is a structural element that constitutes the middle lamellae, wall, and membranes of plant cells; participates in cell division, extension, and compartmentalization; and regulates the action of hormones and signals (Marschner, 1995). Supplementation with increasing Ca has been suggested to increase plant tolerance to salinity stress (Epstein, 1998). Kaya et al. (2002) and Tuna et al. (2007) reported that increased Ca concentrations resulted in enhanced growth and yield of strawberry (*Fragaria ×ananassa* Duch.) and tomato (*Solanum lycopersicon* L.) plants, respectively, exposed to high salinity conditions. The present study was conducted to investigate if supplementary Ca enhances the response of lisianthus to high levels of HCO_3^- -induced alkalinity.

Materials and Methods

Cultural conditions and plant material.

The experiment was conducted in a greenhouse located at Universidad Autónoma Agraria Antonio Narro, in north México (lat. 25°27' N, long. 101°02' W, 1610 m above sea level). Average maximum/minimum temperature were 33/14 °C, and maximum and minimum relative humidity for experiment duration averaged 77% and 64%, respectively. Average photosynthetically active radiation (PAR) measured at solar noon was 361 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Lisianthus cv. ABC Blue plants were transplanted (22 Mar. 2013) into 40-L rigid plastic containers (10 plants per container) with a drainage hole for retrieval of the nutrient solution. Each container was considered an experimental unit and contained 35 L of horticultural-grade perlite [33% water-holding capacity (v/v), 64% air-filled pore space, 0.25 g·cm⁻³ apparent density].

Nutrient solution application. Experimental units were fertigated with nutrient solutions with increasing HCO_3^- -induced alkalinity concentrations: 1, 4, 7 and 10 meq·L⁻¹ of HCO_3^- (from KHCO_3 and NaHCO_3) and two concentrations of Ca: control (8 meq·L⁻¹) and supplementary (11 meq·L⁻¹). Alkalinity was measured by titration three times a week and concentration of HCO_3^- adjusted accordingly by adding NaHCO_3 . The remaining nutrients were supplied according to Steiner's nutrient solution formulation (Steiner, 1984), including macronutrients (meq·L⁻¹): 12 NO_3^- , 1 H_2PO_4^- , 7 K, 4 magnesium (Mg), 2 SO_4^{2-} , and micronutrients (mg·L⁻¹): 5.3 Fe, 0.4 Zn, 2.6 manganese, 0.5 Cu, 0.2 boron (B), and 0.2 molybdenum. Solution pH for solutions with 1, 4, 7, and 10 meq·L⁻¹ HCO_3^- was 6.5, 7.2, 7.7, and 8.5, respectively, whereas electrical conductivity for control and supplementary Ca solutions was 2, 2.3, 2.5, and 2.7 and 2.2, 2.5, 2.6, and 2.9 dS·m⁻¹, respectively. Fertigation was applied through a drip irrigation system with eight emitters per experimental

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unit, and leachate was collected for reuse. Plants were irrigated hourly for 3 min (≈ 1.0 L) from 0800 AM to 1800 HR. Evapotranspired water was replenished daily to each stock tank and the nutrient solutions were replaced every 10 d.

Plant growth and nutrient status. At experiment termination, 90 d after transplanting, growth measurements were recorded in all the plants from each experimental unit, including shoot length (substrate to the top of the inflorescence), diameter at the base of the stem, diameter of flowers (third fully expanded flower), and number of flowers. The plants were separated into roots, stems, leaves, and flowers; washed twice with distilled water; and placed in an oven at 70 °C for 72 h. DM was recorded for each plant part.

Dry shoot tissues were ground to pass a 40-mesh sieve (A-10; Tekmar, IKA Labor-technik, Germany) and digested in a 2:1 mixture of H_2SO_4 : HClO_4 and 2 mL of 30% H_2O_2 . The digested samples were analyzed for nitrogen (N) with micro-Kjeldahl procedure, whereas K, P, Ca, Mg, Cu, Fe, and Zn were analyzed with an inductively coupled plasma emission spectrometer (ICP-AES, model Liberty; VARIAN, Santa Clara, CA).

Photosynthetic parameters, chlorophyll concentration, leaf water potential, and enzymatic activity. Net photosynthetic rate, g_s , and transpiration rate were measured (LI-COR, Inc., Lincoln, NE) at noon on young leaves (the third fully developed leaf from top to bottom) of 90-d-old plants. Average PAR , CO_2 concentration, and temperature were 596 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 364 ppm, and 28.9 °C, respectively. Three measurements on each leaf from one plant per experimental unit were recorded.

Photosynthetic pigments (chlorophyll a, b, and a + b) and catalase and peroxidase activities were measured in young leaves of three plants per replication as described by Jeffrey and Humphrey (1975), Masia (1998), and Kar and Mishra (1976), respectively, at experiment termination. Water potential (Ψ_w) of one young (top third of the plant) and one mature (bottom third of the plant) leaf sampled at solar noon (1200 to 1300 HR) was measured (Scholander Pressure Chamber; Soil Moisture Equipment Corp., Santa Barbara, CA) at experiment termination.

Statistical design. Three replicates of each experimental unit (container with 10 plants) were distributed in a factorial complete randomized block design with four levels of HCO_3^- and two levels of Ca. Data were analyzed using analysis of variance and linear, quadratic, or cubic trend analysis with SAS (SAS Version 8.0; SAS Institute, Cary, NC).

Results

Plant growth. Plants with control Ca concentration exhibited a linear decrease with increasing alkalinity in shoot length (Fig. 1A) and diameter (Table 1), whereas plants with supplemental Ca showed a quadratic response, indicating that supplementary Ca improved plant growth (length) or was unaffected (diameter) even at an alkalinity of 4 $\text{meq}\cdot\text{L}^{-1}$. The

Fig. 1. (A–D) Effect of supplementary calcium (Ca) and bicarbonate (HCO_3^-)-induced alkalinity in the nutrient solution on growth parameters of lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] cv. ABC Blue at experiment termination (90 d after transplanting). Bars represent the SEM ($n = 3$). L, Q, linear, quadratic trends, respectively; NS, *, ** nonsignificant and significant at $P < 0.05$ and < 0.01 , respectively. Alkalinity effect significant for shoot length ($P < 0.001$), total dry mass ($P < 0.001$), leaf dry mass ($P < 0.001$), and root dry mass ($P = 0.002$). Calcium effect significant for root dry mass ($P = 0.004$). Interaction effect significant for total dry mass ($P = 0.044$) and leaf dry mass ($P = 0.033$).

number of flower buds was decreased; however, the decrease was less pronounced in plants with increased Ca because flower bud count was 12% and 34% lower when alkalinity was increased to 4 $\text{meq}\cdot\text{L}^{-1}$ in Ca-supplemented and control plants, respectively (Table 1). The diameter of flowers did not exhibit a consistent tendency (Table 1); however, plants with supplementary Ca produced flowers of similar or larger size than those of the control plants when alkalinity was 7 $\text{meq}\cdot\text{L}^{-1}$ or less.

Total DM of plants (Fig. 1B) as well as leaf (Fig. 1C), stem (Table 1), and flower DM (Table 1) were reduced by increasing alkalinity; however, the deleterious effect of high HCO_3^- was less pronounced in plants that received supplementary Ca when alkalinity was 4 to 7 $\text{meq}\cdot\text{L}^{-1}$. Alkalinity had no effect on root DM of control plants (Fig. 1D), whereas supplementary Ca increased root biomass accumulation regardless of the alkalinity level, except when HCO_3^- was at 10 $\text{meq}\cdot\text{L}^{-1}$.

Physiological parameters. Increasing alkalinity linearly reduced photosynthetic rate of lisianthus regardless of Ca concentration in the nutrient solution (Table 2). Transpiration rate and g_s were unaffected by alkalinity in plants with control Ca concentration; however, in plants with supplementary Ca, transpiration rate increased with 4 $\text{meq}\cdot\text{L}^{-1}$ of HCO_3^- and conductance decreased with increasing concentrations of HCO_3^- (Table 2).

Plants irrigated with supplementary Ca exhibited an association between total DM and g_s [total DM = 202 (g_s) – 34.6; $R^2 = 0.999$]; however, in plants with control Ca concentration, total DM accumulation tended to decrease [total DM = –535 (g_s) + 162, $R^2 = 0.856$]. In control plants, Ψ_w increased in young and mature leaves when exposed to the highest alkalinity (10 $\text{meq}\cdot\text{L}^{-1}$) (Table 2); however, in plants with supplementary Ca, an initial decrease in Ψ_w of both young and mature leaves was detected when exposed to 4 $\text{meq}\cdot\text{L}^{-1}$ of HCO_3^- , which was restored when alkalinity concentration was increased to 7 and 10 $\text{meq}\cdot\text{L}^{-1}$. Increasing alkalinity was associated with a decrease in photosynthetic pigments regardless of Ca concentration (Table 3) and induced an increase in the activity of catalase (Fig. 2A) and peroxidase (Fig. 2B) at both Ca concentrations; however, enzyme activity was higher in plants that received supplementary Ca, especially peroxidase activity, at high alkalinity.

Nutrient status. In both control and plants with supplementary Ca, increasing alkalinity caused a decrease in shoot K, Ca (Table 4), Zn, and Fe (Table 5) concentration, whereas shoot N was affected only in control plants (Table 4). Plants receiving supplementary Ca had a higher concentration of N, K, Ca (Table 4), and Fe (Table 5) when alkalinity of the nutrient solution was 4 $\text{meq}\cdot\text{L}^{-1}$.

Table 1. Effect of calcium (Ca) concentration and bicarbonate (HCO_3^-)-induced alkalinity in the nutrient solution on growth parameters (mean $\pm$ SE, n = 3) of lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] cv. ABC Blue at experiment termination (90 d after transplanting).

HCO ₃ ⁻ (meq·L ⁻¹)	Stem diam (mm)		Flower bud number		Flower diam (cm)		Stem dry mass (g)		Flower dry mass (g)	
	Ca (meq·L ⁻¹)									
	8	11	8	11	8	11	8	11	8	11
1	7.07 ± 0.17	6.94 ± 0.05	15.3 ± 0.94	13.8 ± 0.94	100.9 ± 3.20	99.0 ± 3.55	9.25 ± 0.30	8.53 ± 0.91	6.93 ± 0.61	6.22 ± 0.15
4	6.29 ± 0.30	6.68 ± 0.09	10.1 ± 1.17	12.2 ± 1.04	91.9 ± 0.79	98.8 ± 6.19	5.50 ± 0.75	7.54 ± 0.37	4.14 ± 0.93	5.33 ± 0.16
7	5.79 ± 0.06	5.87 ± 0.43	8.08 ± 0.46	10.9 ± 2.19	100.5 ± 2.80	100.4 ± 4.90	4.66 ± 0.24	5.69 ± 0.93	3.71 ± 0.56	4.68 ± 0.14
10	4.71 ± 0.20	4.67 ± 0.37	6.08 ± 0.73	6.25 ± 1.39	95.2 ± 4.37	88.1 ± 1.72	3.19 ± 0.44	3.08 ± 0.67	2.69 ± 0.49	2.29 ± 0.49
Trend analysis ^z	L**	Q*	Q*	Q*	C*	L*	Q*	Q*	L**	L**
Analysis of variance										
HCO ₃ ⁻	P < 0.001		P < 0.001		P = 0.005		P < 0.001		P < 0.001	
Ca	NS		NS		NS		NS		NS	
Interaction	P = 0.005		P = 0.048		P = 0.047		P = 0.049		NS	

²L, Q, C, linear, quadratic, and cubic trend, respectively.

*, ** Significant at $P < 0.05$ and < 0.01 , respectively.

NS = nonsignificant.

Table 2. Effect of calcium (Ca) concentration and bicarbonate (HCO_3^-)-induced alkalinity in the nutrient solution on selected physiological and water potential (ψ_w) parameters (mean $\pm$ SE, n = 3) of lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] cv. ABC Blue at experiment termination (90 d after transplanting).

HCO_3^- (meq·L ⁻¹)	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$)		Transpiration rate ($\mu\text{g} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$)		Stomatal conductance ($\text{cm} \cdot \text{s}^{-1}$)		Young leaf ψ_w (MPa)		Mature leaf ψ_w (MPa)	
	Ca (meq·L ⁻¹)									
	8	11	8	11	8	11	8	11	8	11
1	12.3 ± 0.65	12.3 ± 0.50	9.45 ± 0.06	9.09 ± 0.19	0.263 ± 0.01	0.270 ± 0.03	-0.926 ± 0.01	-0.910 ± 0.01	-0.910 ± 0.00	-0.873 ± 0.01
4	12.8 ± 0.26	10.3 ± 0.57	9.46 ± 0.65	9.69 ± 0.11	0.283 ± 0.03	0.260 ± 0.05	-0.903 ± 0.01	-0.933 ± 0.00	-0.893 ± 0.01	-0.923 ± 0.01
7	10.3 ± 0.41	9.63 ± 0.28	9.96 ± 0.41	9.31 ± 0.96	0.280 ± 0.03	0.243 ± 0.05	-0.923 ± 0.01	-0.900 ± 0.01	-0.913 ± 0.01	-0.890 ± 0.02
10	8.83 ± 0.11	7.16 ± 0.11	9.99 ± 0.39	9.03 ± 0.37	0.283 ± 0.01	0.210 ± 0.03	-0.856 ± 0.01	-0.873 ± 0.01	-0.883 ± 0.01	-0.893 ± 0.01
Trend analysis ^z	L*	L*	NS	Q*	NS	L*	C**	Q*	NS	C*
Analysis of variance										
HCO_3^-	$P = 0.004$		NS		NS		$P < 0.001$		NS	
Ca	NS		$P = 0.049$		$P = 0.050$		NS		NS	
Interaction	NS		NS		NS		$P = 0.016$		$P = 0.042$	

²L, Q, C, linear, quadratic, and cubic trend, respectively.

NS, *, ** Nonsignificant and significant at $P < 0.05$ and < 0.01 , respectively.

Table 3. Effect of calcium (Ca) concentration and bicarbonate (HCO_3^-)-induced alkalinity in the nutrient solution on chlorophyll concentration (mean $\pm$ SE, n = 3) in young leaves of lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] cv. ABC Blue at experiment termination (90 d after transplanting).

HCO ₃ ⁻ (meq·L ⁻¹)	Chlorophyll a + b (mg·g ⁻¹)		Chlorophyll a (mg·g ⁻¹)		Chlorophyll b (mg·g ⁻¹)	
	Ca (meq·L ⁻¹)					
	8	11	8	11	8	11
1	1.24 ± 0.09	1.21 ± 0.10	0.48 ± 0.04	0.47 ± 0.03	0.77 ± 0.04	0.75 ± 0.05
4	1.19 ± 0.07	1.08 ± 0.07	0.48 ± 0.04	0.39 ± 0.03	0.72 ± 0.07	0.75 ± 0.04
7	0.94 ± 0.03	0.99 ± 0.10	0.33 ± 0.01	0.36 ± 0.04	0.61 ± 0.02	0.64 ± 0.05
10	0.80 ± 0.04	0.80 ± 0.07	0.28 ± 0.02	0.27 ± 0.02	0.52 ± 0.02	0.53 ± 0.04
Trend analysis ^z	L**	L*	L**	L**	L**	L*
Analysis of variance						
HCO ₃ ⁻	<i>P</i> < 0.001		<i>P</i> < 0.001		<i>P</i> < 0.001	
Ca	NS		NS		NS	
Interaction	NS		NS		NS	

²L, Q, C, linear, quadratic, and cubic trend, respectively.

*, ** Significant at $P < 0.05$ and < 0.01 , respectively.

NS = nonsignificant.

Discussion

In the present study, alkalinity in irrigation water reduced the growth of lisianthus; however, plants supplemented with an increased concentration of Ca at alkalinity levels from 4 to 7 meq·L⁻¹ of HCO_3^- exhibited improved growth and DM accumulation or were not detrimentally affected, demonstrating that Ca contributes to the increase of the

tolerance of lisianthus to alkalinity. Alkalinity levels from 4 to 7 meq·L⁻¹ of HCO_3^- have been previously demonstrated to be detrimental to several ornamental species, including roses (*Rosa* sp. L.), chrysanthemum (*Chrysanthemum morifolium* Ramat), ivy geranium [*Pelargonium peltatum* (L.) L'Hér.], and some cultivars of hibiscus (*Hibiscus rosa-sinensis* L.) (Valdez-Aguilar and Reed, 2007).

Photosynthetic rate decreased in plants exposed to increased alkalinity. Supplementary Ca did not ameliorate photosynthetic rate. Supplementary Ca did maintain a high g_s and transpiration rate when alkalinity was at 4 meq·L⁻¹, which may explain the lower ψ_w in young leaves. In plants irrigated with solutions containing supplementary Ca, a higher total DM was associated with a higher g_s ; however, when conductance was higher than

Fig. 2. (A–B) Effect of supplementary calcium (Ca) and bicarbonate (HCO₃⁻)-induced alkalinity in the nutrient solution on catalase and peroxidase activities in lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] cv. ABC Blue at experiment termination (90 d after transplanting). Bars represent the SEM (n = 3). L, Q, C, linear, quadratic, cubic trends, respectively; *, **, *** significant at $P < 0.05$, < 0.01 , and < 0.001 , respectively. Alkalinity effect significant for peroxidase and catalase activity ($P < 0.001$), calcium effect significant for catalase activity ($P = 0.002$), and interaction effect significant for peroxidase activity ($P = 0.018$).

Table 4. Effect of calcium (Ca) concentration and bicarbonate (HCO₃⁻)-induced alkalinity in the nutrient solution on macronutrient concentration (mmol·kg⁻¹) (mean ± SE, n = 3) in shoots of lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] cv. ABC Blue at experiment termination (90 d after transplanting).

HCO ₃ ⁻ (meq·L ⁻¹)	Nitrogen		Phosphorus		Potassium		Calcium		Magnesium	
	Ca (meq·L ⁻¹)									
	8	11	8	11	8	11	8	11	8	11
1	1090 ± 110	1299 ± 118	31.8 ± 1.3	33.3 ± 3.2	516 ± 18	545 ± 47	36.2 ± 1.5	39.9 ± 7.0	117 ± 14	122 ± 20
4	1274 ± 163	1442 ± 133	31.7 ± 3.4	32.6 ± 4.7	422 ± 19	479 ± 26	32.4 ± 3.7	36.1 ± 3.0	115 ± 17	112 ± 12
7	1333 ± 101	1165 ± 66	34.3 ± 1.9	29.7 ± 4.0	413 ± 21	385 ± 34	29.0 ± 5.0	28.3 ± 3.2	102 ± 12	93 ± 13
10	1098 ± 113	956 ± 25	27.6 ± 3.7	24.7 ± 1.9	343 ± 24	342 ± 20	26.7 ± 3.4	25.2 ± 1.7	100 ± 7	82 ± 3
Trend analysis ^z	Q*	NS	NS	NS	L*	L**	L**	L*	NS	NS
Analysis of variance										
HCO ₃ ⁻	NS		NS		P < 0.001		P = 0.004		NS	
Ca	NS		NS		NS		NS		NS	
Interaction	NS		NS		NS		NS		NS	

^zL, Q, linear, quadratic trend, respectively.

NS, *, ** Nonsignificant and significant at $P < 0.05$ and < 0.01 , respectively.

Table 5. Effect of calcium (Ca) concentration and bicarbonate (HCO₃⁻)-induced alkalinity in the nutrient solution on micronutrient concentration (µg·g⁻¹) (mean ± SE, n = 3) in shoots of lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] cv. ABC Blue at experiment termination (90 d after transplanting).

HCO ₃ ⁻ (meq·L ⁻¹)	Zinc		Copper		Iron	
	Ca (meq·L ⁻¹)					
	8	11	8	11	8	11
1	20.8 ± 2.21	28.9 ± 8.07	7.96 ± 1.59	4.48 ± 0.05	54.1 ± 1.81	55.7 ± 1.18
4	17.3 ± 2.83	18.3 ± 3.13	5.42 ± 0.49	5.20 ± 0.75	41.8 ± 7.83	51.1 ± 8.87
7	20.9 ± 1.39	12.9 ± 3.68	4.84 ± 0.38	6.98 ± 1.58	47.5 ± 6.94	35.9 ± 3.06
10	11.2 ± 2.42	11.6 ± 1.51	5.74 ± 0.41	5.46 ± 0.28	33.5 ± 3.45	30.2 ± 3.30
Trend analysis ^z	L*	L*	NS	NS	L*	L**
Analysis of variance						
HCO ₃ ⁻	P = 0.018		NS		P < 0.001	
Ca	NS		NS		NS	
Interaction	NS		P = 0.049		NS	

^zL, linear trend.

NS, *, ** Nonsignificant and significant at $P < 0.05$ and < 0.01 , respectively.

0.280 cm·s⁻¹, like in plants with no supplementary Ca, DM accumulation tended to decrease.

Our results show that alkalinity is associated with plant water relations by increasing ψ_w in young leaves; however, when 8 meq·L⁻¹ of Ca was provided to the plants, there was a disruption on stomata functioning as plants

showed an increase in g_s and transpiration rate that led to DM reduction. The decline in functioning of the stomatal apparatus at high alkalinity was associated with a reduction in shoot K, an ion directly involved in stomata regulation (Marschner, 1995). In plants supplemented with higher Ca, a decrease in shoot K was also detected;

however, Ca ameliorated the uptake of K when alkalinity was 4 meq·L⁻¹ by buffering the reduction in shoot K and Ca. Probably, extracellular Ca may have maintained K content by decreasing K loss through the inhibition of the K outward-rectified channels (Murata et al., 2000). Supplementary Ca and the resulting higher shoot K and Ca

(Atkinson et al., 1990) may have interacted to regulate g_s and thus plant growth.

Chlorophyll concentration is reported to decrease with increasing alkalinity, which has been associated with a decrease in Fe concentration (Roosta, 2011); these findings were corroborated in our experiment. The decline in Fe in the shoot may have been caused by the high pH associated with alkalinity, which renders insoluble forms that plants are unable to uptake (De la Guardia and Alcántara, 2002). In the present study, supplementary Ca increased plant tolerance to alkalinity at 4 meq·L⁻¹ by maintaining a high shoot Fe concentration and a less affected chlorophyll b concentration. Similar results have been reported in white lupine (*Lupinus albus* L.) (Pissaloux et al., 1995). In the present study, chlorophyll b seems to be of greater importance for lisianthus because its concentration tended to be up to 100% higher than that of chlorophyll a.

Compared with plants with control Ca concentration in the nutrient solution, the lower decrease in shoot N, K, Ca, and Fe concentration under high alkalinity (4 meq·L⁻¹) may be the result of the maintenance of membrane integrity when supplementary Ca was supplied. Tuna et al. (2007) and Siddiqui et al. (2013) reported similar results in tomato and radish (*Raphanus sativus* L.) plants when exposed to high salinity and B, respectively, suggesting that the supplementary Ca reduced the electrolytic leakage through cell membranes. Sustaining membrane integrity with Ca may have permitted the normal functioning of channel and carrier proteins and other enzymes such as the Fe-reductase, which would maintain or ameliorate nutrient acquisition under alkalinity stress.

Tolerance to abiotic stress in plants has been connected with the production of reactive oxygen species (ROS) that cause lipid peroxidation in cell membranes and DNA and protein damage (Gill and Tuteja, 2010). In our study, increasing alkalinity caused an increase in catalase and peroxidase activities, indicating that lisianthus responded to alkalinity stress by enhancing enzyme activity to reduce the oxidative damage caused by

ROS. Furthermore, supplementary Ca was associated with a higher catalase and peroxidase activity, probably as a result of its role as a signaling messenger (Reddy and Reddy, 2004; Schmitz et al., 2002) under stressful conditions and providing a complementary mechanism for the tolerance of lisianthus to high alkalinity.

In conclusion, in the present study, supplementary Ca enhanced the tolerance of lisianthus to moderate levels of alkalinity in water (4 meq·L⁻¹) by maintaining 1) an adequate g_s ; 2) an adequate acquisition of N, K, Ca, and Fe; and 3) reducing lipid peroxidation by enhancing the activity of antioxidant enzymes such as peroxidase and catalase.

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