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The Effect of $CaCl_2$ Salinity on Growth Parameters of Lisianthus Cultivars

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Abstract

Soil and water salinity substantially constrain crop and biomass production. To investigate the changes in morphological parameters of lisianthus (*Eustoma grandiflorum*) cultivars under $CaCl_2$ salinity conditions a greenhouse experiment was conducted. Cultivars namely, 'Champagne' (C_1), 'Lime Green' (C_2), 'Blue Picotee' (C_3) and 'Pure White' (C_4), were subjected to salt stress (0–30 mM $CaCl_2$) in a washed sand culture and the morphological responses were measured. The results showed that salinity affected all of the considered parameters, so that, as salinity levels increased, plant height, shoot fresh and dry weight and leaf area in all cultivars decreased, while, root length, root fresh and dry weight and root: shoot length ratio increased. However, the changes in 'Pure White' and 'Lime Green' were less than in 'Champagne' and 'Blue Picotee'. The regression analysis of the relationship between $CaCl_2$ salinity levels and seedlings height or root: shoot length ratio defined two groups with different slope coefficients: C_1 and C_3 as salt sensitive cultivars and C_2 and C_4 as salt tolerant cultivars. The results showed that salinity threshold of C_1 and C_3 was 25 and 30 mM $CaCl_2$ respectively, while C_2 and C_4 in 30 mM $CaCl_2$ showed no significant differences with control. The results suggests that C_2 and C_4 could be recommended as resistant cultivars due to attain higher growth, water balance, shoot fresh and dry weight and leaf area in response to $CaCl_2$ salinity compared with C_1 and C_3.

Keywords: $CaCl_2$-Salinity, *Eustoma grandiflorum*, Resistant cultivars, Threshold.

INTRODUCTION

Various types of environmental stress such as drought, nutrient imbalances, and salinity may unfavorably affect the growth, development and productivity of crops. Salinity is one of the most important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (Zhao *et al.*, 2007). Salt stress severely depresses a wide range of physiological metabolism and biochemical reactions, therefore, can adversely affect plant growth due to the interrupt certain morphological and physiological processes lead to reductions in plant growth and quality. Plant growth, leaf area and productivity reduce with increasing salinity due to certain types of ionic toxicity, resulting a decrease in the ability of plants to uptake water from the soil solution (Morales *et al.*, 1998; Valdez-Aguilar *et al.*, 2014). To mitigate the detrimental effects of salinity, searching for and using salt tolerant genotypes can be applied (Ashraf and Ahmad, 2000; Turhan and Seniz, 2010). Plant salt tolerance is the ability to resist the effects of high salt concentrations in the root zone without significant adverse effects. Salt tolerance is usually assessed as the percent biomass production in saline conditions versus a control (Munns, 2002). Because of the increased need for food production and increasing distribution of soils affected by salinity, and water scarcity, the use of salt tolerant species in agriculture, particularly for landscaping projects and cut flowers production, should be considered (Navarro *et al.*, 2008). A number of studies have been conducted to investigate the degree of salt tolerance and the associated mechanisms in different crops, such as sunflower (Akram *et al.*, 2009; Shahbaz *et al.*, 2011), olive (Chartzoulakis *et al.*, 2002) and ornamental shrubs (Cassaniti *et al.*, 2009).

Lisianthus (*Eustoma grandiflorum*) has become a consumer favorite in the cut flower market because of its exceptional blooms and long vase life (Valdez-Aguilar *et al.*, 2014). Many cultivars of lisianthus used for cut flowers show morphological variations in flower color, size and shape (Shimizu-Yumoto and Ichimura, 2010). Studies of plant tolerance to salt stress cover many aspects of the influences of salinity on plant behavior, including alterations at the morphological, physiological and molecular levels. Some families of floral crops, such as Asteraceae have been found to exhibit salt tolerant qualities. However, information on morphological, ornamental and physiological changes provoked by the saline irrigation conditions is lacking for lisianthus and the tolerances of cultivars remain poorly understood. The goal of this study was to achieve a better understanding of the effect of $CaCl_2$ salinity in irrigation water on growth parameters of lisianthus cultivars. As part of this study, the collected cultivars were evaluated for their $CaCl_2$ salinity resistant using growth, dry and fresh weight as selection criteria related to salt tolerance.

MATERIALS AND METHODS

The experiment was conducted in a research greenhouse at the Faculty of Agriculture, Lorestan University, Iran (32°37'N, 46°51'E) during 2015. Day and night air temperatures ranged from 22–32°C and 16–20°C, respectively. The experiment was done as factorial arrangement based on completely randomized design with four replications. Seven salinity levels including: 0 (control), 5, 10, 15, 20, 25 and 30 mM $CaCl_2$ were added to half strength Hoagland's nutrient solution.

The salinity levels were tested on four lisianthus cultivars namely 'Champagne' (C_1),' Lime Green' (C_2), 'Blue Picotee' (C_3) and 'Pure White' (C_4) (fig. 1). F_1 hybrid seeds of the cultivars were obtained from Sakata Seed Company (Japan) and were sown in trays filled with cocopeat and perlite. The resulting seedlings were selected for shoot uniformity, and transferred into pots (one seedlings per pot). Washed sand was used as the potting mixture and cultural practices were applied regularly. Seedlings were allowed to establish by fertigating them twice a day (at 09:00 and 14:00 h) with half-strength Hoagland's nutrient solution that contained: 2.5 mM Ca $(NO_3)_2$, 0.2 µM $CuSO_4$, 40 µM Fe (as Fe-EDTA), 23 µM $H3BO_3$, 0.5 mM KH_2PO_4, 2.5 mM KNO_3, 1.0 mM $MgSO_4$, 4.5 µM $MnCl_2$, 0.2 µM Na_2MoO_4 and 0.4 µM $ZnSO_4$. The pH of the nutrient solution was adjusted to 5.8 ± 0.1 and the electrical conductivity (EC) was 1.7 ± 0.1 dS m^{-1}. One week after transplanting, salinity treatments were started with 5 mM $CaCl_2$. Seedlings were fertilized with nutrient solution containing $CaCl_2$ twice a day until flowering (approximately 70 days after transplanting).

Fig. 1. Four lisianthus cultivars used in this experiment.

Growth characters were recorded in all seedlings from each experimental unit, including seedlings height (from medium surface to the top of the seedlings), leaf area, fresh and dry weight of both shoot and root. The seedlings were harvested and separated into roots and shoots and their fresh weight were measured. The seedlings materials were washed, then were placed in an oven at 70°C for 72-h. dry weight was recorded for each seedlings part. Leaf area were measured using a DT-scan leaf area meter (Delta T-scan, Version 2.03; Delta -T Devices Ltd., Burwell, Cambridge, UK).

Data analysis

The data were subjected to analysis of variance using SAS statistical software (Version 9.1; SAS Institute, Cary, NC, USA). Mean comparisons were done according to the Least Significant Difference (LSD) at $P<0.05$. The slopes of linear regressions between the $CaCl_2$ concentrations and seedlings height of lisianthus cultivars were calculated and tested using Graph Pad Prism to verify if slopes of the two regression lines were statistically different ($P<0.05$).

RESULTS

The results showed that cultivars effect was significant for all parameters except leaf area. Also salinity had effect on all morphological parameters of lisianthus except root fresh and dry weight (Table 1). The results showed that all morphological parameters in 'Lime Green' and 'Pure White' was higher than 'Champagne' and 'Blue Picotee' except of leaf area and stem diameter that was greater in 'Champagne' compared with other cultivars (Table 2). Leaf number in 'Lime Green' was the highest, while, 'Blue Picotte' had the lowest leaf number. Also, results showed that leaf number and leaf area was decreased as salinity increases. Base on this results, as $CaCL_2$ salinity levels increased, seedlings shoot fresh and dry weight of tested cultivars decreased in high concentration of $CaCl_2$. In contrast, the response of root length, fresh weight and dry weight of lisianthus cultivars to salinity was different, and increased with increasing salinity (Table 3).

Table 1. Analysis of variance data related to the effect of salinity on some morphological characteristics of lisianthus cultivars.

S.o.V	df	Mean Square (MS)									
		Height	Leaf number	Leaf area	Root fresh weight	Root dry weight	Root length	Flower bud diameter	Shoot fresh weight	Shoot dry weight	diameter
Cultivar (C)	3	395.75***	705.28**	62.57^{ns}	12.95**	1.25**	913.93**	1.18^{ns}	51.51**	0.326^{ns}	3.09**
Salinity (S)	6	49.09^{ns}	58.5*	286.2*	3.842^{ns}	0.133^{ns}	104.29*	7.22*	14.9**	0.636**	0.394**
C × S	18	30.53*	31.33^{ns}	82.39^{ns}	2.84^{ns}	0.257*	50.8^{ns}	3.69^{ns}	3.32^{ns}	0.086^{ns}	0.186*
Error	81	25.22	21.04	109.97	1.71	0.119	42.09	2.99	3.84	0.129	0.099
CV (%)		13.76	18.04	26.6	26.97	36.4	18.59	29.18	24.75	29.49	11.56

^{ns}: Non significant, *: Significant at 5% level probality, **: Significant at 1% level probality.

Table 2. Simple effect of cultivar on some morphological characteristics of lisianthus cultivars.

Cultivars	Leaf number ($plant^{-1}$)	Leaf area (cm^2)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)	Flower bud diameter (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Stem diameter (mm)
C_1	26.39 b	8.32 a	4.35 b	0.719 b	28.83 c	6.08 b	7.38 b	1.16 ab	3.01 a
C_2	29.25 a	7.78 a	6.08 a	1.24 a	44.14 a	6.47 ab	9.42 a	1.37 a	2.41 c
C_3	18.10 c	7.50a	4.90 b	0.76 b	32.02 bc	5.88 b	5.92 c	1.07 b	2.84 b
C_4	27.96 ab	7.90 a	4.54 b	1.06 a	35.32 b	7.16 a	8.82 a	1.21 ab	2.32 c
LSD	2.43	1.29	0.83	0.203	4.03	0.742	1.21	0.224	0.177

Mean values in each column followed by the same lower-case letters did not differ significantly at $P<0.05$ according to LSD test. C_1: Champagne; C_2: Lime Green; C_3: Blue Picotee; C_4: Pure White.

Table 3. Simple effect of CaCl2 salinity on some morphological characteristics of four lisianthus cultivars.

Salinity of $CaCl_2$ (mM)	Leaf number ($plant^{-1}$)	Leaf area (cm^2)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)	Flower bud diameter (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Stem diameter (mm)
0	27.9 a	8.78 a	4.81 abc	0.780 b	31.7 bc	5.81 cd	8.88 a	1.32 ab	2.41 b
5	28.3 a	6.68 b	4.23 c	0.960 ab	31.16 c	6.55 abc	8.32 a	1.58 a	2.82 a
10	24.6 b	7.87 ab	4.90 abc	0.873 ab	33.70 abc	6.91 ab	8.59 a	1.32 ab	2.52 b
15	25.1 ab	8.47 a	4.37 bc	0.838 ab	35.62 abc	7.23 a	8.52 a	1.21 bc	2.92 a
20	24.4 b	8.79 a	5.37 ab	0.981 ab	36.54 ab	6.79 abc	8.29 a	1.09 bcd	2.81 a
25	23.8 b	6.44 b	5.29 abc	0.990 ab	37.87 a	6.20 bcd	6.49 b	1.029 cd	2.51 b
30	23.6 b	7.91 ab	5.82 a	1.088 a	38.95 a	5.22 d	6.10 b	0.884 d	2.54 b
LSD	3.22	1.71	1.09	0.272	5.33	0.981	1.6	0.291	0.227

Mean values in each column followed by the same lower-case letters did not differ significantly at $P<0.05$ according to LSD test.

The results showed that as salinity levels increased, plant height of 'Champagne' and 'Blue Picotte' cultivars decreased, while, plant height of 'Pure White' and 'Lime Green' increased (Fig.2).

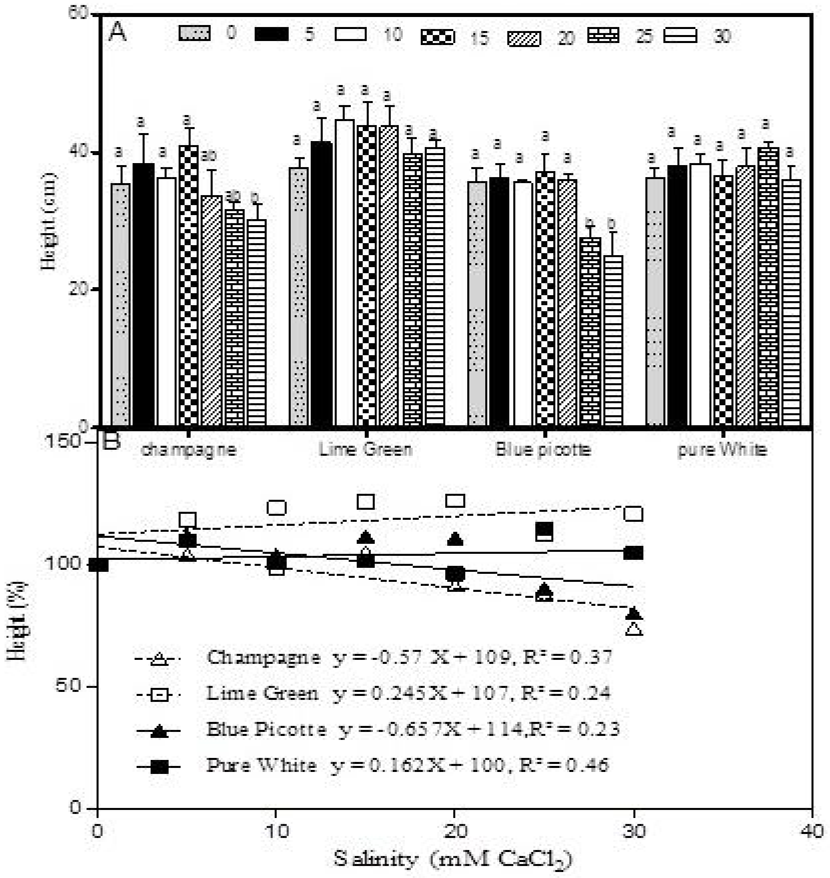

Fig. 2. The effect of $CaCl_2$ salinity levels on plant height (A: Absolute values, B: Linear regression) of four lisianthus cultivars.

However, the decline in plant height of 'Blue Picotee' and 'Champagne' started at 25 mM $CaCl_2$ respectively, while, plant height of 'Lime Green' and 'Pure White' in 30 mM $CaCl_2$ had no significant differences compared with control. The regression analysis of the relationship between salinity levels and plant height showed two groups with different slope coefficients: The 'Blue Picotee' and 'Champagne' cultivars with an average slope of −0.61, and the 'Lime Green' and 'Pure White' cultivars with an average slope of −0.2. Within group, slope coefficients of cultivars were not differ significantly, while, there was a significant difference ($P<0.05$) between the two groups (Fig. 2B). The increased $CaCl_2$ levels caused a decline in shoot dry weight in 'Champagne' and 'Blue Picotte' cultivars, while, shoot dry weight of 'Pure White' and 'Lime Green' showed no significant decreases as salinity levels increased (Fig. 3). Similar results was obtained in regression analysis of shoot fresh weight in lisianthus cultivars.

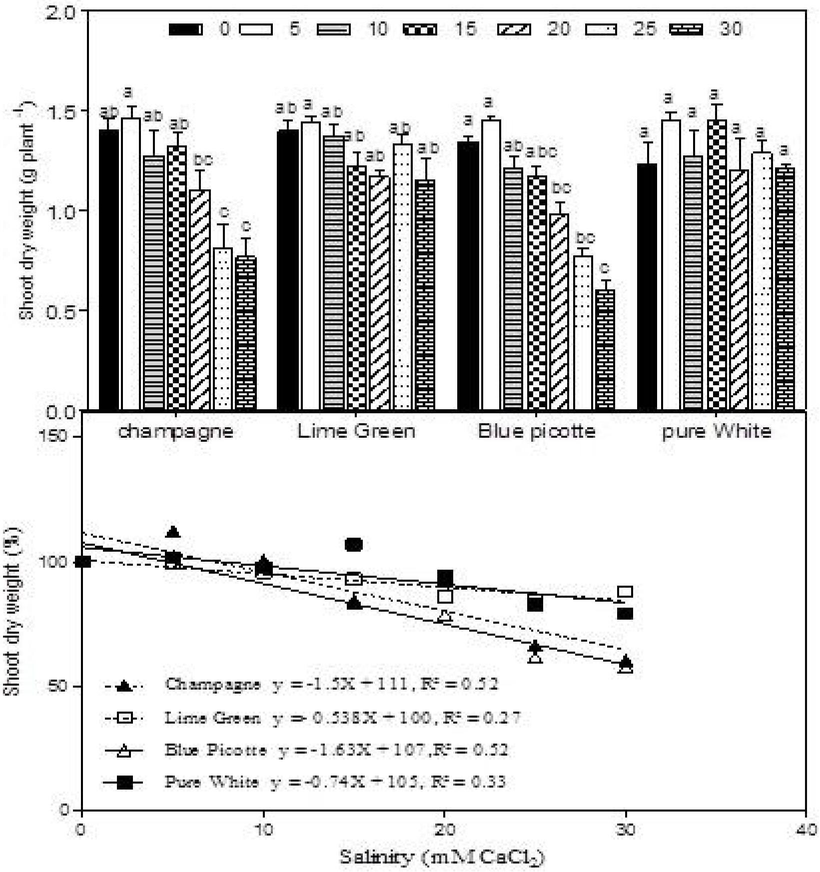

Fig. 3. The effect of $CaCl_2$ salinity levels on shoot dry weight (A: Absolute values, B: Linear regression) of four lisianthus cultivars.

The root: shoot length ratio increased proportionally to the $CaCl_2$ salinity levels of the nutrient solution (Fig. 4). Similar to the plant height versus EC response function, the root-to-shoot length ratio versus $CaCl_2$ salinity also defined two groups with different slope coefficients: The 'Blue Picotee' and 'Champagne' cultivars with an average slope of 0.022, and the 'Lime Green' and 'Pure White' cultivars with an average slope of 0.0085. Within group, slope coefficients of cultivars were not differ significantly, while, there was a significant difference ($P < 0.05$) between the two groups (Fig. 4).

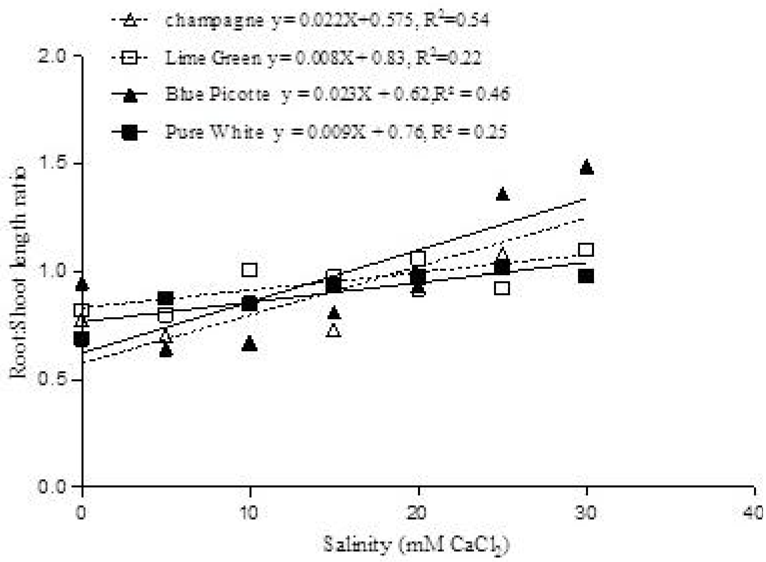

Fig. 4. The effect of $CaCl_2$ salinity levels on root: shoot length ratio of four lisianthus cultivars.

DISCUSSION

Salinity is one of the serious environmental problem that cause osmotic stress and reduction in plant growth and crop productivity in irrigated areas of arid and semiarid regions (Cassaniti *et al.*, 2009; Munns and Tester, 2008). The main effect of salinity on glycophytes is to reduce growth and this reduction has been used in many studies as a measure of resistance to salinity (Cassaniti *et al.*, 2009; Chartzoulakis *et al.*, 2002; Shahbaz *et al.*, 2011). Wide variation was observed in tolerance to salt stress among lisianthus cultivars tested here. According to our results, $CaCl_2$ treatments caused a reduction in seedlings growth parameters. Similar results by NaCl salinity have been reported previously (Ashrafi and Nejad, 2017; Hafsi *et al.*, 2007; Maggio *et al.*, 2007; Pérez-Tornero *et al.*, 2009; Sairam *et al.*, 2002; Tarchoune *et al.*, 2012). In the present research, the growth parameters of all cultivars was reduced by $CaCl_2$ salinity, albeit the decrease in the C_1 and C_3 appeared to be greater compared with C_2 and C_4.

These CUL exhibited large differences in salt tolerance; however, sensitive cultivars in the present cultivars study responded to salinity at 25 mM $CaCl_2$ (equivalent 6.5 dS m^{-1}). It has been reported previously that lisianthus ‘Raf Shinn’ could be grown profitably when irrigated with saline water with an EC as high as 8 dS m^{-1}, without measurable effects (Valdez-Aguilar *et al.*, 2013). The differences could be related to different responses of cultivars to salinity. In the present research, root fresh weight increased in lisianthus cultivars tested here as salinity increased (Table 2). Increase of root biomass in response to salinity have been reported previously (Ashrafi and Nejad, 2017; Maggio *et al.*, 2007; Thitisaksakul and Maysaya, 2008). This has been attributed to the reallocation of photosynthesis materials into the roots more than shoots (Saab *et al.*, 1990; Thitisaksakul and Maysaya, 2008). Further, our results revealed that root to shoot length ratio increased with increasing salinity in all cultivars, however, sensitive cultivars showed larger root to shoot length ratio compared with tolerant cultivars (Fig. 4). This could be due to higher decline of shoot length in sensitive cultivars in response to salinity compared with that in tolerant cultivars (Fig. 2).

It is evident from the data presented here that by increasing the $CaCl_2$ supply, in low concentration, height and other growth parameters increased. The Ca^{2+} is a moderator of the effects of stress, because it maintains the integrity of cell membranes and is a cofactor for several enzymes. It has been shown that Ca^{2+} protects membranes from the adverse effects of reactive oxygen species (ROS), thereby maintaining its integrity and minimizing destructive (López-Pérez *et al.*, 2014;

Rengel, 1992). Resistance to biotic or abiotic stress imparted by the $CaCl_2$ has been associated with a temporary increase in intracellular Ca^{2+} concentration, which acts as a second messenger in the generation of adaptive to counteract the harmful effects responses (Batistič and Kudla, 2010). The $CaCl_2$ treatment enhanced different H_2O_2 scavenging enzymes, such as Superoxide dismutase, peroxidase, catalase, and non-enzymatic antioxidants. This enhancement would have helped in scavenging of ROS in lisianthus plants. These findings indicate that the suppressive effect of salinity on plant growth had at least partly an ion specific origin and were not depending exclusively on level of salinity. Harmful effect of $CaCl_2$ salinity may be due to high Cl^- concentration and also physiological drought caused by salinity.

CONCLUSION

According the results presented here, significant differences in salt tolerance were observed among the lisianthus cultivars, implying that these differences could be considered when selecting salt tolerant and sensitive cultivars. C_2 and C_4 could be recommended as resistant cultivars due to higher growth parameters in response to salinity compared with C_1 and C_3.

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