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Lisianthus response to salinity stress

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Abstract

The effect of salinity on some morpho-physiological characteristics in lisianthus cultivars was investigated. Cultivars namely, Blue Picotee (C_1), Champagne (C_2), Lime Green (C_3), and Pure White (C_4), were subjected to salt stress (0–60 mM NaCl) in a sand culture and their responses were measured. Our results showed that as a salinity level increased, growth parameters, relative water content, photosynthetic pigments, and gas-exchange characteristics decreased in all cultivars, while root fresh mass, root/shoot length ratio, electrolyte leakage, and a malondialdehyde content increased. However, the changes were less pronounced in C_3 and C_4 compared to C_1 and C_2. The regression analysis of the relationship between salinity levels and seedling height or root/shoot length ratio defined two groups with different slope coefficients: C_1 and C_2 as salt-sensitive cultivars and C_3 and C_4 as salt-tolerant cultivars. Shoot dry mass and leaf area tolerance indices were less affected by salinity in C_3 and C_4 compared to those in C_1 and C_2. Further, C_3 and C_4 showed higher photosynthetic rates, greater stomatal conductances, and accumulated greater K^+ and Ca^{2+} contents and K^+/Na^+ ratios in roots and shoots compared to those in C_1 and C_2. The results suggests that C_3 and C_4 could be recommended as resistant cultivars due to maintaining higher growth, water balance, leaf gas exchange, ion compartmentalization, and lower lipid peroxidation in response to salinity compared to C_1 and C_2.

Additional key words: cultivar; gas exchange; NaCl-salinity; tolerance index.

Introduction

Currently, one third of all irrigated lands is affected by salinity worldwide and salinity stress remains one of the most serious environmental problems limiting crop production (Munns 2005, Turhan and Şeniz 2010). Irrigation with saline water introduces salt into the soil, resulting in a decrease of osmotic potential in root environment, thus making it difficult for roots to extract water from soils (Rengasamy 2006). Plant growth and productivity decline with increasing salinity due to interruption of certain morphological and physiological processes and certain types of ionic toxicity and nutritional imbalances (Morales *et al.* 1998, Valdez-Aguilar *et al.* 2014). Also, salinity reduces plant quality and marketability, and represents a significant challenge to ornamental horticultural production systems.

There are several strategies to cope with salinity problem in plant production, including improvements of irrigation methods, such as drip and subsurface irrigation system, reclamation of salinized lands, and other special horticultural techniques. However, these techniques are expensive, difficult to apply, and take a very long time to have an effect (Turhan and Şeniz 2010). While improvement of soil and water management can help to solve the problem, obtaining salt-tolerant cultivars is one of the most effective strategies to cope with salinity (Flowers and Yeo 1995, Essa 2002, Ruiz-Carrasco *et al.* 2011). Therefore screening salt tolerance in plants, particularly for landscaping projects and cut flowers production, is crucial for recommendations of suitable plants (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*), native to the arid zones of the southern United States and northern Mexico, (Gómez-Pérez *et al.* 2014), is an ornamental plant of increasing demand. Lisianthus has become a consumer favorite in the cut flower market because of its exceptional

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Abbreviations: C_1 – Blue Picotee; C_2 – Champagne; C_3 – Lime Green; C_4 – Pure White; Chl – chlorophyll; DM – dry mass; EL – electrolyte leakage; E – transpiration rate; FM – fresh mass; g_s – stomatal conductance; LA – leaf area; MDA – malondialdehyde; P_N – net photosynthetic rate; RWC – relative water content; TI – tolerance index.

blooms and long vase life (Shimizu-Yumoto and Ichimura 2010). There are many lisianthus cultivars with morphological variations in flower color, size, and shape (Shimizu-Yumoto and Ichimura 2010). Tolerance to salt appears to be cultivar-dependent. Despite some interesting papers dealing with the environmental conditions required for lisianthus production, cultivar responses to NaCl salinity has not been extensively investigated. A recent report on lisianthus "Raf Shinn" showed salinity tolerance up to 8 dS m^{-1} (Valdez-Aguilar *et al.* 2013). However, salinity responses in other cultivars have not been tested. Therefore, the objective of the present study was to achieve a better understanding of the effect of irrigation with saline water on different lisianthus cultivars. The main focus of this study was the comparative study on NaCl salinity tolerance using growth parameters, photosynthetic attributes, and accumulation of ions in roots and shoots.

Materials and methods

Experimental site: 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 between 22–32°C and 16–20°C, respectively. Relative air humidity ranged between 55–65% and average daily PAR was 400–500 μmol(photon) m^{-2} s^{-1}.

Experimental design and salinity treatments: The experiment was done with factorial arrangement based on a completely randomized design (CRD) with four replications. Seven salinity levels including: 0 (control), 10, 20, 30, 40, 50, and 60 mM NaCl (corresponding to electrical conductivity (EC) of 1.7, 2.94, 4.14, 5.35, 6.25, 7.36, and 8.45 dS m^{-1}, respectively) were added to half-strength Hoagland solution. The salinity levels were tested on four lisianthus cultivars (C_1, C_2, C_3, and C_4). F_1 hybrid seeds of the cultivars were obtained from *Sakata Seed Company* (Japan) and were sown in trays filled with cocopeat and perlite. Uniform seedlings were transferred into pots (one seedling 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 nutrient solution containing 2.5 mM Ca $(NO_3)_2$, 0.2 μM $CuSO_4$, 40 μM Fe (as Fe-EDTA), 23 μM H_3BO_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 EC was 1.7 ± 0.1 dS m^{-1}. One week after transplanting, salinity treatments were started with 10 mM NaCl and to avoid salt shock the concentration were increased by 10 mM per day to reach the desired salinity levels. Seedlings were fertilized with nutrient solution containing NaCl twice a day until flowering (approximately 70 d after transplanting).

Growth and tolerance indices (TIs): Growth characteristics were recorded in all seedlings from each experimental unit, including seedling height (from substrate surface to the top of the seedling), leaf area (LA), fresh mass (FM), and dry mass (DM) of both shoots and roots. The seedlings were harvested and separated into roots and shoots and their FM were measured. DM was recorded after oven-drying at 70°C for 72 h. LA was measured using a leaf area meter (*Delta T-scan*, *Version 2.03; Delta-T Devices Ltd*., *Burwell*, Cambridge, UK).

TI was calculated according to (Shi and Cai 2009) using the following equation:

$$TI = [100 \times (T_x / T_0)]$$

where T_x is the value of the parameter as determined for stressed seedlings, and T_0 is the value of the parameter as determined for control seedlings.

Ion concentrations: Dry tissues were ground to pass through a 40-mesh sieve, digested with 1.0 M hydrochloric acid, and then Ca^{2+}, Cl^-, K^+, and Na^+ concentrations in roots and shoots were determined (Mills *et al.* 1996). K^+ and Na^+ concentrations were determined by flame photometry (*PFP7*; *Jenway*, *Chelmsford*, Essex, UK). Chloride ion concentrations were determined by titration with $AgNO_3$ in the presence of K_2CrO_4 (Anonymous 1915) and Ca^{2+} was determined using ethylenediaminetetraacetic acid (EDTA) (Walsh and Douglas 1972).

Gas-exchange parameters were measured on four fully expanded mature leaves, 60 and 70 d after treatments, using a portable infrared gas exchange analyser (*LCA4*, *ADC Bioscientific, Ltd*., Hoddesdon, England). Due to large number of seedlings for gas-exchange measurements, each replicate was measured for all treatments (all salinity levels and cultivars) randomly in one day from 9:30 to 11:30 h and in total all measurement were completed in four successive days. The conditions of gas-exchange measurement were: leaf surface area of 6.25 cm^2, ambient CO_2 concentration (C_{ref}) of 350 μmol mol^{-1}, temperature of leaf chamber (T_{ch}), relative humidity, and PAR at leaf surface varying from 26–29°C, 58–62%, and 420–460 μmol(photon) m^{-2} s^{-1}, respectively. Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) measurements were carried out under steady-state conditions (about 60 s).

Physiological and biochemical characteristics: Leaf chlorophyll (Chl *a*, Chl *b*, and Chl total) concentrations were determined according to Lichtenthaler (1987), using a spectrophotometer (*U-1800 UV/VIS, Hitachi*, Japan). Relative water content (RWC) was obtained from fully expanded leaves as (FM – DM)/(FSM – DM) × 100, where

FM is fresh mass, FSM is fresh saturated mass after rehydrating samples for 24-h in the dark, and DM is dry mass after oven-drying samples at 70°C to constant mass. Electrolyte leakage (EL) was measured following the method of Lutts *et al.* (1996) to assess membrane permeability using an electrical conductivity meter. Lipid peroxidation was estimated by measuring the formation of malondialdehyde (MDA) according to Wang *et al.* (2009).

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 $P \leq 0.05$. The slopes of linear regressions between the NaCl concentrations and seedlings height of lisianthus cultivars were calculated and tested using *GraphPad Prism 5* to verify if slopes of the two regression lines were statistically different ($P \leq 0.05$).

Results

Seedling height: The results showed that as salinity level increased, seedling height of all tested cultivars decreased (Fig. 1*A*). However, the decline in seedling height of C_1 and C_2 started at 20 mM NaCl, while, seedling height of C_3 and C_4 was reduced at 50 and 40 mM NaCl, respectively. The regression analysis of the relationship between salinity levels and seedling height (% of control values) showed two groups with different slope coefficients: C_1 and C_2 with an average slope of –0.87, and C_3 and C_4 with an average slope of –0.49. Within a group, slope coefficients of cultivars were not different significantly, while, there was a significant difference ($P<0.05$) between the two groups (Fig. 1*B*).

Root and shoot FM: As salinity level increased, shoot FM decreased in all cultivars (Fig. 2). In contrast, the response of root FM to salinity was different in each cultivar. In C_2, C_3 and C_4 root FM increased with increasing salinity, while, root FM of C_1 did not show any clear change in response to salinity.

The root/shoot length ratio increased with increasing NaCl concentration (Fig. 3). Similarly to the seedling height, the root/shoot length ratio *vs*. salinity also defined two groups with different slope coefficients: C_1 and C_2 cultivars with an average slope of 0.023, and C_3 and C_4 with an average slope of 0.0093. Within a group, slope coefficients of cultivars were not different significantly, while, there was a significant difference ($P<0.05$) between the two groups.

TI: Shoot DM and LA TIs of C_1 and C_2 were more affected by salinity than those of C_3 and C_4 (Fig. 4). In addition, root K^+/Na^+ TI was higher in C_3 than that of the other cultivars. Shoot K^+/Na^+ TI was lower in C_1 and C_2 than that in C_3 and C_4.

Element concentrations: As the salinity level increased, the concentration of Cl^- and Na^+ increased, while, Ca^{2+} and K^+ contents and K^+/Na^+ ratio decreased (Table 1). C_3 and C_4 accumulated greater K^+ and Ca^{2+} contents in roots and shoots than those of C_1 and C_2. Further, shoot K^+/Na^+ ratios in C_3 and C_4 were higher than those of C_1 and C_2. A strong positive correlation was found between shoot K^+ content and shoot FM ($r = 0.61$, $P<0.0001$). In addition, there were significant negative correlations between both root and shoot Na^+ with shoot K^+ contents ($r = –0.39$, $P = 0.0002$ and $r = –0.46$, $P<0.0001$ for roots and shoots, respectively).

Gas-exchange characteristics: P_N, E, and g_s decreased with increasing NaCl concentration (Table 2). For example, P_N of seedlings grown under 60 mM NaCl on 60 and 70 d after treatment decreased by 51 and 41%, respectively, compared with control. Moreover, C_4 showed the highest P_N, followed by C_1 and C_3, and the lowest P_N was found in C_2. C_4 showed higher E than in the other cultivars, while, the highest g_s was found in C_3.

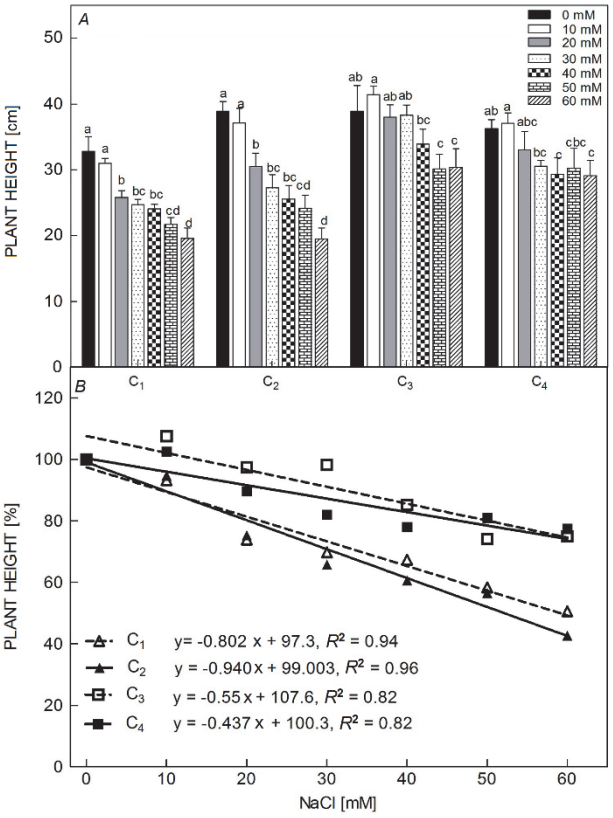

Fig. 1. The effect of salinity levels on seedling height (*A*: absolute values, *B*: linear regression) of four lisianthus cultivars. C_1 – Blue Picotee, C_2 – Champagne, C_3 – Lime Green, C_4 – Pure White. $P_{cultivar}<0.0001$, $P_{salinityr}<0.0001$, $P_{cultivar \times salinity}= 0.258$.

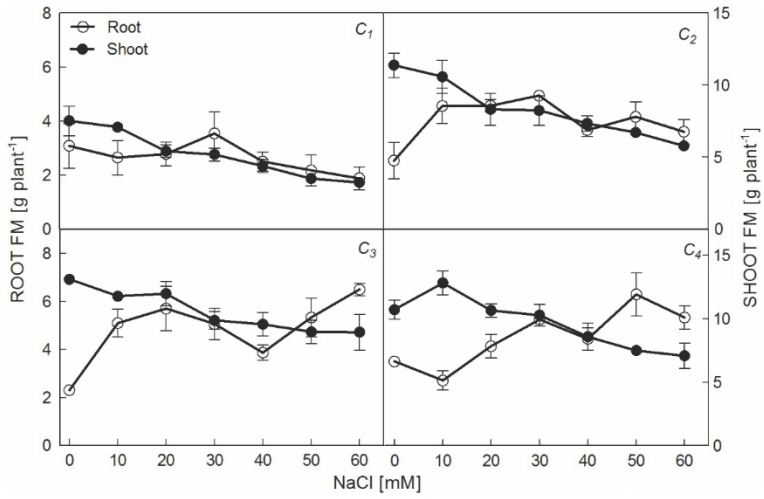

Fig. 2. Changes of root and shoot fresh mass (FM) of lisianthus cultivars at different salinity concentrations. C_1 – Blue Picotee, C_2 – Champagne, C_3 – Lime Green, C_4 – Pure White. Root FM: $P_{cultivar}<0.0001$, $P_{salinity}=0.029$, $P_{cultivar\times salinity}=0.185$; shoot FM: $P_{cultivar}<0.0001$, $P_{salinity}<0.0001$, $P_{cultivar\times salinity}=0.686$.

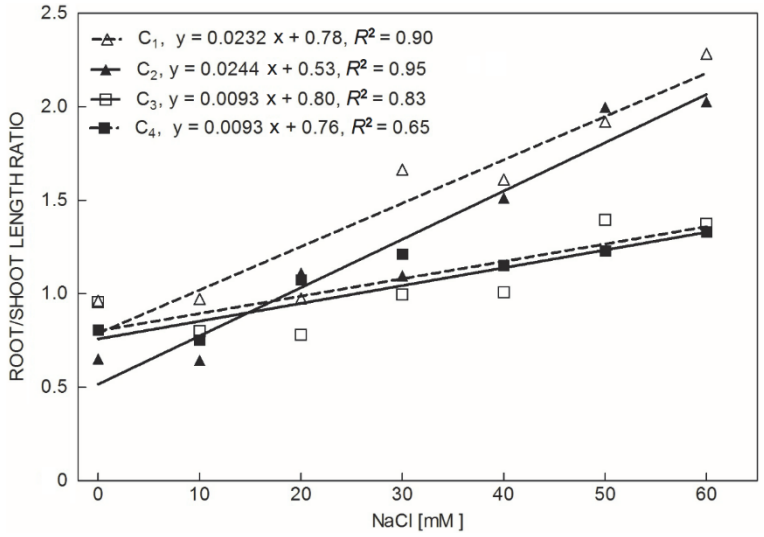

Fig. 3. The effect of salinity levels on root/shoot length ratio of four lisianthus cultivars. C_1 – Blue Picotee, C_2 – Champagne, C_3 – Lime Green, C_4 – Pure White.

Membrane lipid peroxidation: Salinity impaired membrane permeability as shown by larger EL and MDA content in seedlings grown under higher salinity levels compared with controls (Table 3). However, C_1 and C_2 were more sensitive than C_3 and C_4.

Leaf Chl content decreased with the increasing NaCl concentration. C_3 and C_4 showed higher Chl contents compared to C_1 and C_2 (Table 3).

RWC: Lower RWCs were found in seedlings treated with higher NaCl concentrations. Moreover, C_4 showed the highest RWC, followed by C_1 and C_3, and the lowest RWC was found in C_2 (Table 3).

Discussion

The general pattern of plant responses to salinity shows a growth suppression depending on salt concentrations and plant species, and these responses have been used in many studies as a measure of resistance to saline conditions (Chartzoulakis *et al.* 2002, Cassaniti *et al.* 2009, Shahbaz *et al.* 2011). According to our results, NaCl treatments reduced seedling growth due to decreased P_N, g_s, and RWC and increased EL and MDA content. Similar results have been reported previously (Sairam *et al.* 2002, Hafsi *et al.* 2007, Maggio *et al.* 2007, Pérez-Tornero *et al.* 2009, Tarchoune *et al.* 2012). In current research, the growth parameters of all cultivars were reduced by salinity, though, the decrease in the C_1 and C_2 appeared to be greater than that in C_3 and C_4. It has been reported that lisianthus 'Raf Shinn' could be grown profitably when irrigated with saline water with an EC of 8 dS m^{-1}, without measurable effects (Valdez-Aguilar *et al.* 2013). However, sensitive and resistant cultivars in the present study responded to salinity at 20 and 50 mM NaCl (equal to 4.14 and 7.36 dS m^{-1}), respectively. The differences could be related to different responses of cultivars to salinity.

In the present research, root FM in three out of four tested cultivars increased as salinity increased (Fig. 2). Increased root biomass in response to salinity have been

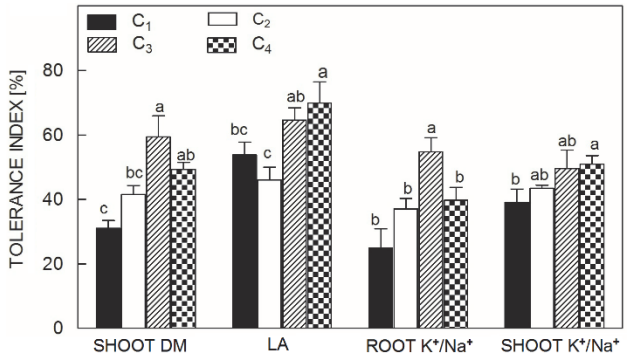

Fig. 4. Tolerance indices (TI) of shoot dry mass (DM), leaf area (LA), root and shoot K+/Na+ ratio in control and 60 mM NaCl-treated seedlings of four lisianthus cultivars. C_1 – Blue Picotee, C_2 – Champagne, C_3 – Lime Green, C_4 – Pure White. Shoot DM: P=0.0018; LA: P=0.015; root K^+/Na^+: P=0.0062; shoot K^+/Na^+: P=0.0018.

reported previously (Maggio *et al.* 2007, Pattanagul and Thitisaksakul 2008). This has been attributed to the reallocation of photosynthates into roots more than shoots (Saab *et al.* 1990, Pattanagul and Thitisaksakul 2008). Further, our results revealed that root/shoot length ratio increased with increasing salinity, however, sensitive cultivars (C_1 and C_2) showed larger root/shoot length ratio compared to tolerant cultivars (C_3 and C_4) (Fig. 3). This could be due to higher decline of shoot length in sensitive cultivars in response to salinity compared to that in tolerant cultivars (Fig. 1).

The analysis of TI was used as a simple and effective method to select salt-tolerant cultivars at different salt concentrations. Cultivars with higher TI, showing lesser decreases in seedlings height and biomass could be more tolerant than those with lower TI (Turhan and Şeniz 2010, Sharma *et al.* 2013). The results of current study showed

Table 1. Effect of NaCl salinity on the concentration of some nutrient elements [mg g^{-1}] in the shoots and roots of four lisianthus cultivars. C_1 – Blue Picotee; C_2 – Champagne; C_3 – Lime Green; C_4 – Pure White. Mean values in each column followed by the same *lowercase letters* did not differ significantly at $P \leq 0.05$ according to LSD.

Treatment	Cl^- Root	Ca^{2+}	Na^+	K^+	K^+/Na^+	Cl^- Shoot	Ca^{2+}	Na^+	K^+	K^+/Na^+
Cultivar										
C_1	3.84^a	0.44^b	9.2^a	5.5^c	0.69^a	2.26^a	0.21^c	12.7^a	7.8^b	0.65^b
C_2	4.03^a	0.45^b	9.9^a	5.4^c	0.58^b	2.14^a	0.25^b	12.3^{ab}	8.2^b	0.71^b
C_3	3.82^a	0.52^a	10.08^a	6.8^a	0.70^a	2.23^a	0.30^a	11.9^{ab}	9.7^a	0.86^a
C_4	4.01^a	0.51^{ab}	10.1^a	6.05^b	0.64^{ab}	2.23^a	0.31^a	11.4^b	10.01^a	0.9^a
LSD	0.45	0.07	0.98	0.55	0.103	0.48	0.04	0.57	0.98	0.102
NaCl [mM]										
0	2.9^c	0.72^a	6.5^e	6.9^a	1.11^a	1.56^d	0.26^b	9.4^d	10.1^a	1.10^a
10	3.54^b	0.73^a	8.7^d	6.4^{abc}	0.73^b	1.74^{cd}	0.33^a	9.9^d	9.6^{ab}	0.98^a
20	3.44^{bc}	0.42^{bc}	9.58^{cd}	6.6^{ab}	0.7^{bc}	2.08^{cd}	0.37^a	12.03^c	9.6^{abc}	0.81^b
30	3.74^b	0.46^b	10.1^{bc}	5.8^{cd}	0.58^{cd}	1.89^{cd}	0.26^b	12.8^{bc}	8.9^{bc}	0.71^{bc}
40	3.9^b	0.41^{bc}	10.9^{ab}	5.9^{bc}	0.55^{de}	2.36^{bc}	0.187^c	13.3^{ab}	8.8^{cd}	0.67^c
50	5.0^a	0.35^{cd}	11.04^{ab}	5.09^{de}	0.47^{de}	3.01^a	0.24^b	13.2^{abc}	8.1^{de}	0.67^c
60	4.8^a	0.29^d	11.9^a	4.94^e	0.42^e	2.87^{ab}	0.244^{bc}	14.1^a	7.4^e	0.53^d
LSD	0.6	0.092	1.3	0.73	0.136	0.63	0.76	0.05	1.29	0.135
$P_{cultivar}$	0.703	0.056	0.272	<0.0001	0.071	0.966	0.0001	0.052	<0.0001	<0.0001
$P_{salinity}$	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
$P_{cultivar \times salinity}$	0.317	0.45	0.241	0.782	0.081	0.989	0.256	0.057	0.532	0.128

that C_3 and C_4 were more tolerant than C_1 and C_2, as they showed higher growth (Figs. 1, 2, 3) and TIs (Fig. 4).

Reduced growth under saline conditions could be partly due to high concentrations of salt accumulated in the growing tissues and low absorption of nutrient ions, *i.e.*, Ca^{2+} or K^+ (Sharma *et al.* 2013). All cultivars tested here, tended to decrease K^+ and Ca^{2+} contents and to increase Na^+ and Cl^- contents in response to increasing salinity level (Table 1). This could be due to the antagonistic effect of Na^+ on K^+ and Ca^{2+} absorption caused by a decreased water potential in the root environment, as reported previously (Mavrogianopoulos *et al.* 2002, Sairam *et al.* 2002, Turhan and Şeniz 2010). Lower accumulation of toxic ions (*i.e.*, Na^+ and Cl^-), and higher capacity for Ca^{2+} or K^+ uptake in seedlings tissues resulting in higher K^+/Na^+ ratio, are characteristics of tolerant cultivars (Poustini and Siosemardeh 2004, Izadi *et al.* 2014). C_3 and C_4 in the present research were able to maintain a constant K^+ content independently of Na^+ and Cl^- accumulation (Table 1), suggesting an efficient salt compartmentalization. Further, K^+/Na^+ ratio in tolerant cultivars was higher than that of the sensitive ones. Similar findings have been reported on barley and wheat (Izadi *et al.* 2014) and lettuce (Tarakcioglu and Inal 2002).

Based on the results, salinity reduced P_N, g_s, and E (Table 2). The effect of salinity on P_N could be through

Table 2. Effect of NaCl salinity on leaf gas exchange of four lisianthus cultivars after 60 and 70 days from treatment. C_1 – Blue Picotee; C_2 – Champagne; C_3 – Lime Green; C_4 – Pure White; E – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate. Mean values in each column followed by the same *lowercase letters* did not differ significantly at $P \leq 0.05$ according to LSD.

Treatment	P_N [μmol m^{-2} s^{-1}]		E [mmol(H_2O) m^{-2} s^{-1}]		g_s [mol(H_2O) m^{-2} s^{-1}]	
	Time after treatment [d]					
	60	70	60	70	60	70
Cultivar						
C_1	2.6^{b}	2.5^{b}	2.9^{b}	3.6^{b}	0.128^{bc}	0.135^{b}
C_2	1.8^{c}	2.01^{c}	2.8^{b}	3.6^{b}	0.115^{c}	0.136^{b}
C_3	3.1^{a}	2.5^{ab}	3.2^{ab}	3.7^{b}	0.156^{a}	0.182^{a}
C_4	3.4^{a}	2.9^{a}	3.8^{a}	4.2^{a}	0.149^{ab}	0.139^{b}
LSD	0.414	0.46	0.533	0.35	0.021	0.021
NaCl [mM]						
0	3.7^{a}	2.9^{a}	4.09^{a}	4.4^{ab}	0.184^{a}	0.167^{ab}
10	3.8^{a}	2.7^{ab}	3.5^{ab}	4.01^{bc}	0.166^{ab}	0.188^{a}
20	2.6^{bc}	3.2^{a}	3.3^{bc}	4.68^{a}	0.141^{bc}	0.178^{a}
30	2.9^{b}	2.8^{a}	2.8^{cd}	3.82^{cd}	0.123^{cd}	0.147^{bc}
40	2.1^{cd}	2.16^{bc}	2.9^{bcd}	3.42^{de}	0.132^{c}	0.12^{cd}
50	2.0^{d}	1.9^{c}	3.09^{bcd}	3.3^{ef}	0.115^{cd}	0.117^{d}
60	1.8^{d}	1.7^{c}	2.6^{d}	2.9^{f}	0.098^{d}	0.116^{d}
LSD	0.547	0.61	0.706	0.47	0.027	0.02
$P_{cultivar}$	<0.0001	0.0012	0.0022	0.0025	<0.0001	<0.0001
$P_{salinity}$	<0.0001	<0.0001	0.0026	<0.0001	0.001	<0.0001
$P_{cultivar \times salinity}$	0.0215	0.298	0.93	0.0127	0.208	0.064

Table 3. Effect of NaCl salinity on some physiological and biochemical characteristics of four lisianthus cultivars. C_1 – Blue Picotee; C_2 – Champagne; C_3 – Lime Green; C_4 – Pure White ; Chl – chlorophyll; EL – electrolyte leakage; MDA – malondialdehyde; RWC – relative water content. Mean values in each column followed by the same *lowercase letters* did not differ significantly at $P \leq 0.05$ according to LSD.

Treatment	El [%]	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Chl total [mg g^{-1}(FM)]	MDA [μmol g^{-1}(FM)]	RWC [%]
Cultivar						
C_1	32.2^{b}	16.7^{ab}	5.4^{b}	23.2^{b}	4.53^{a}	74.25^{bc}
C_2	38.1^{a}	16.5^{b}	5.3^{b}	21.8^{b}	4.69^{a}	71.09^{c}
C_3	26.4^{c}	19.4^{a}	7.7^{a}	27.2^{a}	2.9^{b}	77.39^{ab}
C_4	21.5^{d}	18.6^{ab}	7.6^{a}	25.2^{ab}	2.81^{b}	82.13^{a}
LSD	3.53	2.82	1.50	3.81	0.626	4.98
NaCl [mM]						
0	24.6^{c}	20.03^{a}	8.2^{a}	28.2^{a}	2.73^{e}	80.68^{ab}
10	26.4^{bc}	17.8^{ab}	6.6^{abc}	24.4^{abc}	3.17^{de}	83.24^{a}
20	25.8^{bc}	19.7 ^{a}	7.4^{ab}	27.1^{ab}	3.48^{cde}	74.48^{bcd}
30	28.4^{bc}	15.38^{b}	5.1^{c}	20.5^{c}	4.31^{ab}	78.6^{abc}
40	29.8^{b}	17.1^{ab}	5.9^{bc}	23.09^{bc}	4.05^{abc}	74.86^{bc}
50	34.6^{a}	18.01^{ab}	6.08^{bc}	24.09^{abc}	3.59^{bcd}	73.61^{cd}
60	37.1^{a}	16.8^{ab}	6.3^{abc}	23.2^{bc}	4.8^{a}	68.02^{d}
LSD	4.68	3.73	1.98	5.04	0.828	6.59
$P_{cultivar}$	<0.0001	0.185	0.010	0.040	<0.0001	0.0004
$P_{salinity}$	<0.0001	0.152	0.087	0.068	0.0001	0.0006
$P_{cultivar \times salinity}$	0.0001	0.720	0.830	0.734	0.165	0.154

reduced CO_2 partial pressure in the leaves due to stomatal closure (Meloni *et al.* 2003, DeRidder and Salvucci 2007, Maksimović *et al.* 2010) and/or nonstomatal factors, such as (*1*) a decline in photosynthetic pigments as showed by our results (Table 3) and previous reports (Tabatabaei and Ehsanzadeh 2016), and (*2*) a disturbance in homeostasis of Na^+ and Cl^- ions and essential mineral nutrients (Dionisio-Sese and Tobita 2000, Gunes *et al.* 2007). Moreover, lower E may be explained by a decrease in g_s in the presence of high NaCl concentrations (Jia *et al.* 2002, Maksimović *et*

al. 2010). However, the response of cultivars to salinity in terms of leaf gas-exchange parameters was different. While the highest P_N and E were found in C_4, the largest g_s was found in C_3. Further studies may be required to find an explanation for different cultivar responses in leaf gas-exchange characteristics to salinity stress.

Cell membrane permeability has long been used as an effective selection criterion for salinity tolerance in plants (Farooq and Azam 2006). Peroxidation of membrane lipids (which is measured by EL and MDA content) is an indication of membrane damage and leakage under salt stress conditions (Katsuhara *et al.* 2005). EL and MDA content increased as salinity level increased in all cultivars (Table 3). However, C_1 and C_2 showed higher EL and MDA contents compared to C_3 and C_4, suggesting that, oxidative damage induced by salt was more pronounced in these cultivars compared to C_3 and C_4.

RWC decreased as salinity level increased (Table 3). The early response of seedlings to salinity is lowering leaf water potential and RWC. The decrease in RWC is a result of high salt concentration of the external solution, which caused osmotic stress and dehydration at cellular level (Chartzoulakis 2005). Bongi and Palliotti (1994) reported that a reduction in RWC caused a reduction in P_N. Cultivars which can limit the accumulation of harmful elements and maintain a positive water balance in their tissues are more tolerant to salt. Our data indicated that, C_3 and C_4 grown under salinity showed lower reduction in RWCs compared with those in C_1 and C_2 suggesting that C_3 and C_4 cultivars can tolerate greater concentrations of salts than C_1 and C_2.

Conclusions: In the present study, salt stress reduced growth, RWC, photosynthetic pigments, and gas-exchange characteristics, while it enhanced root FM, root/shoot length ratio, EL and MDA content in all cultivars. According the results presented here, significant differences in salt tolerance were observed between the lisianthus cultivars, implying that these differences could be considered when selecting salt tolerant and sensitive cultivars. C_3 and C_4 could be recommended as resistant cultivars due to higher growth, water balance, leaf gas exchange, ion compartmentalization, and lower lipid peroxidation in response to salinity compared to C_1 and C_2.

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