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Tolerance mechanisms of three potted ornamental plants grown under moderate salinity

Pedro García-Caparrós[a], Alfonso Llanderal[a], Maribela Pestana[b], Pedro José Correia[b], María Teresa Lao[a,*]

[a] Higher Polytechnic School and Experimental Science College, Department of Agronomy of the University of Almeria, Agrifood Campus of International Excellence ceiA3. Ctra. Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain
[b] Universidade do Algarve, MeditBio, FCT, Edifício 8, Campus de Gambelas, 8005-139 Faro, Portugal

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ABSTRACT

The scarcity of water in the Mediterranean area has frequently led to the use of saline water in order to irrigate ornamental plants in many nurseries. However, before the large-scale use of such waters, the ways in which the plants deal with the salinity need to be evaluated. Plants of *Aloe vera* L. Burm, *Kalanchoe blossfeldiana* Poelln and *Gazania splendens* Lem sp. were grown in pots with a mixture of sphagnum peat-moss and Perlite. In order to evaluate the effects of different levels of salinity, three treatments using different NaCl concentrations (Electrical conductivity = 2.0 (control), 4.5 and 7.5 dS m^{-1}) were applied over a period of 60 days. At the end of the experiment, the growth, physiological parameters and mineral content of the roots and leaves were assessed for each salinity treatment. After 60 days of exposure to salinity, the total biomass of all species decreased similarly. The mineral composition of roots and leaves was clearly affected. Osmolytes, such as proline, played an important role in the osmotic adjustment in all species increasing in the roots and leaves at the higher EC_i. Different mechanisms of the salt tolerance were triggered in each species. *A vera* plants showed Na^+ accumulation at the root level and a decrease in succulence index of leaves. *K. blossfeldiana* plants shed leaves to release Na^+ and *G. splendens* plants accumulated Cl^- and Na^+ at the root level, secreted salt from leaves, lost salt by shedding of old leaves and increased the succulence index of remaining leaves. We concluded that the use of saline waters is feasible for growing these ornamental plants, and *G. splendens* seems to be particularly well adapted to salinity, a consideration that is particularly relevant in arid saline areas.

1. Introduction

The worldwide production value of ornamental potted plants and cut flowers is about 50 billion €, corresponding to an estimated global consumption of between 100 and 150 billion € (Lütken et al., 2012). However, nowadays there is a decrease in production around the world partly due to soil and water salinization (Cassaniti et al., 2013) as happens in the south-eastern coastal region of Spain with high salinity levels in the water due to the overexploitation of groundwater and seawater intrusion in some aquifers (Consejería de Medio Ambiente y Ordenación del Territorio (CMAOT), 2012).

There are three major constraints for plant growth under saline conditions: (1) the water stress, arising from lower water potential of the growing medium, (2) ion toxicity associated with the excessive uptake mainly of Cl and Na, and (3) a nutrient imbalance caused by depression in the uptake of other nutrient ions (Marschner, 1995). To cope with the effects of salt stress, plants have evolved many biochemical and molecular mechanisms to reduce detrimental effects of ions from those parts of the plants where they may be harmful; these mechanisms include accumulation at the root level, the shedding of dry leaves, salt secretion and succulence (Aslam et al., 2011). The cell osmotic adjustment necessary for growth in saline environments may be accomplished by the accumulation of inorganic and organic solutes. The inorganic ions are believed to

Abbreviations: ANOVA, analysis of variance; DW, dry weight; EC_i, electrical conductivity of the irrigation water; FW, fresh weight; H_2O_2, hydrogen peroxide; HPLC, high performance liquid cromatography; LWR, leaf weight ratio; LSD, least significant difference; L, leaves; PAR, photosynthetically active radiation; RH, relative humidity; RWR, relative root weight ratio; Yr, relative yield; R, roots; SI, succulence index; H_2SO_4, sulphuric acid; TDW, total dry weight; TSS, total soluble sugars; UL, unwashed leaves; WL, washed leaves; WC_l, water content in leaves; WC_r, water content in roots; Y, yield.

* Corresponding author.
E-mail addresses: pedrogar123@hotmail.com (P. García-Caparrós), mtlao@ual.es, mtlaoa@yahoo.es (M.T. Lao).

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be sequestered in the vacuoles, while the organic solutes such as sugars and proline may be compartmentalized in the cytoplasm to balance the low osmotic potential in the vacuole (Munns and Tester, 2008).

Due to the great economic importance of the production of potted plants in south-eastern coast of Spain in the recent years, and the challenges to the continued supply of non-saline water mentioned above, further investigation is necessary on the effects of different irrigation water salinity (electrical conductivity, EC_i) in horticultural species. The adjustment of the nutrient solution in terms of electrical conductivity (EC) is crucial for the optimization of the water and nutrient availability (Kang and Iersel, 2004).

We investigated three species. *Aloe vera* L. Burm, a member of the *Asphodelaceae* family, is a succulent plant with green leaves, widely cultivated and valued due to its short growth period and the high economic value (Moghbeli et al., 2012). *Kalanchoe blossfeldiana* Poelln, originating from Madagascar, is a member of *Crassulaceae* (Abdel-Raouf, 2012) and it is one of the most financially important flowering, potted plant species in Europe, with a production of more than 150 million plants per year (Mibus et al., 2014). *Gazania splendens* Lem a sp., within Compositae, tribe *Arctotideae*, subtribe *Gorteriinae* (Karis, 2007), is an ornamental shrub widely cultivated in gardens across the world, being endemic from southern Africa (Magee et al., 2011). Nevertheless, very few studies on the effects of different EC of the irrigation water on the nutrition and physiology have been reported for *K. blossfeldiana* (Taybi et al., 1995; Mariaux et al., 1997) and *G. splendens*. In the case of *A. vera*, there are many investigations on the effects of high NaCl concentration such as 100 and 200 mM NaCl (Xu et al., 2006; Zheng et al., 2009) or 100% seawater (Liu et al., 2007), but very little is known regarding the effects under low NaCl stress. Therefore, in this trial, a pot experiment with *A. vera*, *K. blossfeldiana* and *G. splendens* plants was established in order to determine the effects of different salinity levels of the irrigation water on the plants' dry mass and allocation, mineral nutrient content, mechanisms of salt tolerance and their physiological changes. Such information can be used for optimizing the crop management with saline waters and also for evaluating which of these species might be suitable for the use of saline waters.

2. Material and methods

2.1. Plant material and experimental conditions

The present study was carried out at the University of Almeria (36°49′N, 2°24′W). Rooted cuttings (plants) of *A. vera* L. Burm, *K. blossfeldiana* Poelln and *G. splendens* Lem were obtained from a local nursery and transplanted into 1.5 L polyethylene pots containing a mixture of sphagnum peat-moss and Perlite 80:20 (v/v). During the trial (60 days), the pots were placed in a greenhouse of 150 m^2. The microclimatic conditions inside the greenhouse for the experimental period, monitored continuously with HOBO SHUTTLE sensors (model H 08-004-02) showed a daily average temperature of 25.4 ± 2.5 °C, relative humidity (RH) of 65.6 ± 2.1% and photosynthetically active radiation (PAR) of 225 ± 9.4 μmol m^{-2} s^{-1}.

2.2. Experimental design and treatments

This experiment had been performed previously with a wider range of salinities. The experiment consisted of three treatments using different salinities in a standard solution with the following composition (in mmol L^{-1}): 0.70 $H_2PO_4^-$, 6.00 NO_3^-, 2.00 SO_4^{2-}, 3.00 K^+, 2.00 Ca^{2+} and 1.40 Mg^{2+} amended with different concentrations of NaCl (sole salinizing agent) to achieve EC levels of the irrigation water (EC_i) of either 2.0 (T_1 or control, 3 mmol L^{-1} NaCl), 4.5 (T_2, 30 mmol L^{-1} NaCl) or 7.5 (T_3, 60 mmol L^{-1} NaCl) dS m^{-1}. The plants were irrigated manually every day. The EC_i and pH were measured daily using a conductivity meter and pH meters (models Milwaukee C66 and pH52), respectively. The treatments (EC levels) were chosen in accordance with previous research reported by Wu and Dodge (2005) regarding the salinity tolerance with a range from 2 to over 6 dS m^{-1} to avoid salt stress symptoms, considering the irrigation with the nutrient solution of 2.0 dS m^{-1} as a control in the experiment. The volume of saline water added to each pot during the experimental growing period was 4.2 L for each saline treatment and the same for all species. The experimental design consisted of three salinity treatments, four blocks, and four plants (one plant per pot) per block giving a total of 12 plants per species plus border plants.

2.3. Plant parameters

At the end of the saline period, the plants were harvested and the substrate gently washed from the roots of four plants per treatment for all the studied species. The plants were divided into roots (R) and leaves (L) and the respective fresh weights (FW) measured; roots and leaves were then oven-dried at 60 °C until they reached a constant weight to measure the respective dry weights (DW). These dry weights were used to calculate several plant parameters as indicated by Ryser and Lambers (1995) and Correia et al. (2010): the leaf weight ratio (LWR; leaf DW per unit plant DW) and the relative root weight ratio (RWR; root DW per unit plant DW). The total dry weight (TDW) was calculated as the sum of leaves and roots DW. The fresh and dry weight of roots and leaves were used to calculate the water content (WC) (−) as indicated by Ben Amor et al. (2005):

$$WC = \frac{(FW - DW)}{FW} \quad (1)$$

2.4. Yield response salinity models

To model the yield response to the different EC_i values in the three species, regression analyses were tested and the best fitted models were selected based on the determination coefficient (R^2) in accordance with Steppuhn et al. (2005) and Correia et al. (2010). In this experiment, the total plant DW (roots and leaves) was used as yield (Y), being assessed 60 days from salinization. The absolute yield (Y) was converted into relative yield (Yr) by employing a scaling divisor (Ym) based on the maximum value of total plant biomass (DW) obtained in control plants (Maas and Hoffman, 1977). The Yr value for each salt treatment was determined at the end of the experiment according to the following equation:

$$Yr = \frac{Y}{Ym} \quad (2)$$

2.5. Physiological measurements

Four plants per treatment were randomly selected at the end of the experiment in each species to determine the Na and Cl accumulation by roots, calculated as the ratio between the quantity of Cl and Na in the root (R) relative to total quantity in the plant (Cl^- and Na^+ extraction per plant in mmol/root DW in gram); (ii) the Cl^- and Na^+ secretion by leaves (L) was assessed by the difference between the content of this elements in washed leaves (WL) and in unwashed leaves (UL) (mmol g^{-1} DW); (iii) the loss of Na and Cl was evaluated by collecting and quantifying the contents of these elements (mmol g^{-1} DW) in shed old leaves, and (iv) the succulence index (SI) of leaves was determined as the ratio between leaf FW and leaf DW (−) as proposed by Hoolbrook and Putz (1996).

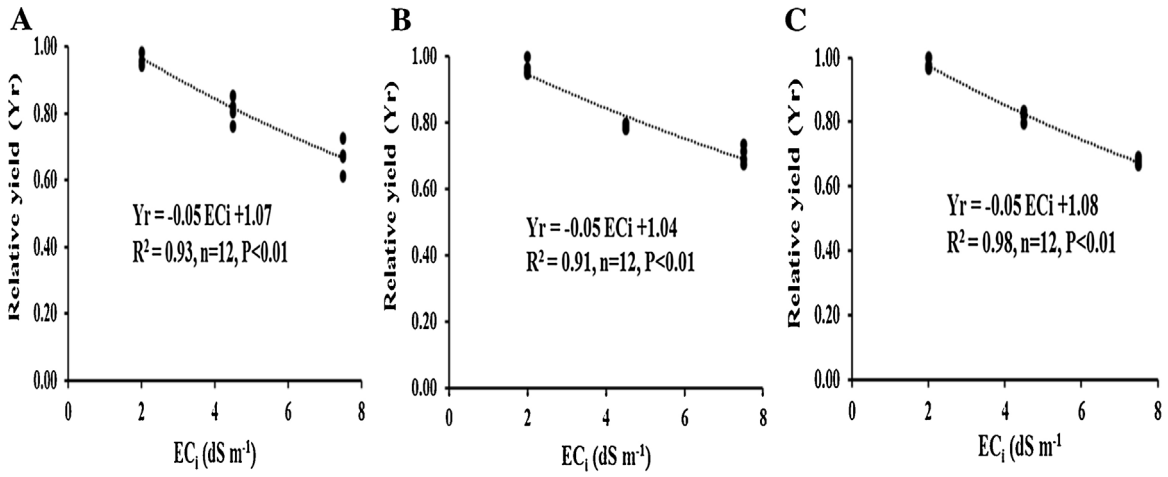

Fig. 1. Relationship between the relative yield (Yr: expressed as TDW) and the EC_i in *A. vera* (A), *K. blossfeldiana* (B) and *G. splendens* (C) plants at the end of the experiment.

2.6. Analysis of mineral elements

The oven-dried samples were ground in a mill and divided in two subsamples. The analysis of mineral elements of the roots and leaves was determined in one subsample following water extraction by HPLC (High Performance Liquid Chromatography; model Metrohm 883 Basic IC Plus). The soluble ionic forms (NO_3^- and Cl^-) were quantified using a column model Metrosep A SUPP 4 (IC conductivity detector range 0–15000 $\mu S\,cm^{-1}$) as described by Csáky and Martínez-Grau (1998). The mobile phase was prepared by mixing 190.6 mg of CO_3^{2-} and 142.8 mg of HCO_3^- and then diluting in 1 L of deionized water, acidify with H_2SO_4 (50 mM). The other subsample was mineralized with sulphuric acid (H_2SO_4, 96%) in the presence of hydrogen peroxide (H_2O_2, P-free) at 300 °C and used for the determination of organic N, total P, K^+ and Na^+ concentration. To determine the organic N concentration (Krom, 1980), 1 mL of reagent A (8.5 g of sodium salicylate and 0.06 g of sodium nitroprusside in 100 mL of deionized water) and 1 mL of reagent B (4 g of sodic hydroxid and 0.625 g of sodic dicloroisocinurate in 100 mL of deionized water) were added to 0.1 mL of mineralized, shaking afterwards. Passed 45 min, the absorbance at 630 nm was quantified colorimetrically (model Shimadzu UV-1201) comparing with a standard curve of $(NH_4)_2SO_4$. For the determination of total P (Hogue et al., 1970), the phosphorus reagent was prepared as follows: 10 g of ammonium molybdate and 5 g of ammonium vanadate were added to 800 mL of boiling deionized water. Then, the solution was cooled in ice and 4 mL of nitric acid were added drop by drop. Afterwards, 134 mL of nitric acid were transferred by burette, diluting with deionized water to the mark in a 1 L volumetric flask and mixing. Finally, 0.5 mL of mineralized, 1.5 mL of deionized water and 3 mL of phosphorus reagent were added, mixing and allowing to stand for 1 h afterwards. The absorbance was quantified colorimetrically at 430 nm (model Shimadzu UV-1201) using a calibration curve prepared with K_2HPO_4 standard solution. The total N was calculated as the sum of the organic N and NO_3^- concentration. The total K^+ and Na^+ concentrations were directly measured in the mineralized by flame spectrophotometry (model Jenway PFP 7) (Lachica et al., 1973).

2.7. Organic solutes determinations

To determine the concentrations of proline and total soluble sugars (TSS) in the roots and leaves, four plants were randomly selected per treatment in each species at harvest. Fresh material (0.5 g for each type of organ) was crushed in 5 mL of 95% (v/v) ethanol and centrifuged (model Digicen 21 R) at 3500 × *g* for 10 min. The pellet was washed twice with 5 mL of 70% (v/v) ethanol and recentrifuged. The free proline and TSS concentrations were determined in the alcoholic extract supernatant. The free proline concentration was determined by the ninhydrin reagent method. A volume of 2.5 mL of ninhydrin reagent (25 g of ninhydrin mixed with 400 mL of phosphoric acid 6 M and 600 mL of 60% (v/v) glacial acetic acid shaken at 75 °C) plus 4 mL of distilled water and 2.5 mL of glacial acetic acid were added to 2 mL of alcoholic extract supernatant, shaken and heated in a boiling water-bath for 45 min. The reaction was stopped by placing the test tubes in an ice bath. Then 5 mL of benzene were added to the samples, being vigorously mixed in a vortex shaker for 1 min afterwards. After the extraction with benzene, the free proline concentration was quantified colorimetrically at 515 nm using L-proline (Sigma Chemicals) as standard. Free proline concentration was expressed as $\mu mol\,g^{-1}$ FW. The total soluble sugars concentration was determined by the anthrone reagent method. Four mL of anthrone reagent (300 mg of anthrone mixed with 300 mL of 70% (v/v) sulphuric acid) was added to 100 μL of alcoholic extract supernatant and then the mixture was shaken, heated in a boiling water-bath for 10 min and cooled at 4 °C. Lastly, the total soluble sugars concentration was quantified colorimetrically at 650 nm using glucose (Sigma Chemicals) as standard. The total soluble sugars concentration was expressed as μmol glucose g^{-1} FW (Irigoyen et al., 1992).

2.8. Statistical analysis

The experiment had a completely randomized block design, and the values obtained for each plant and each variable were considered as independent replicates. Each species was analysed independently. The data were analysed through one-way analysis of variance (ANOVA) and least significant difference (LSD) tests ($P < 0.05$) in order to assess the differences between treatments using Statgraphic Plus for Windows (version 5.1.).

3. Results

3.1. Plant parameters

Throughout the experiment, there were no mortalities in any of the species in response to salinity treatments (Table 1). Salinity decreased the total dry weight (TDW) in all the species (root dry weight (RDW) was unaffected by EC_i whereas leaf dry weight (LDW) decreased significantly by salinity). The leaf weigh ratio (LWR) was not affected due to salinity but the values of relative root weight ratio (RWR) were lower in the control compared to salinized

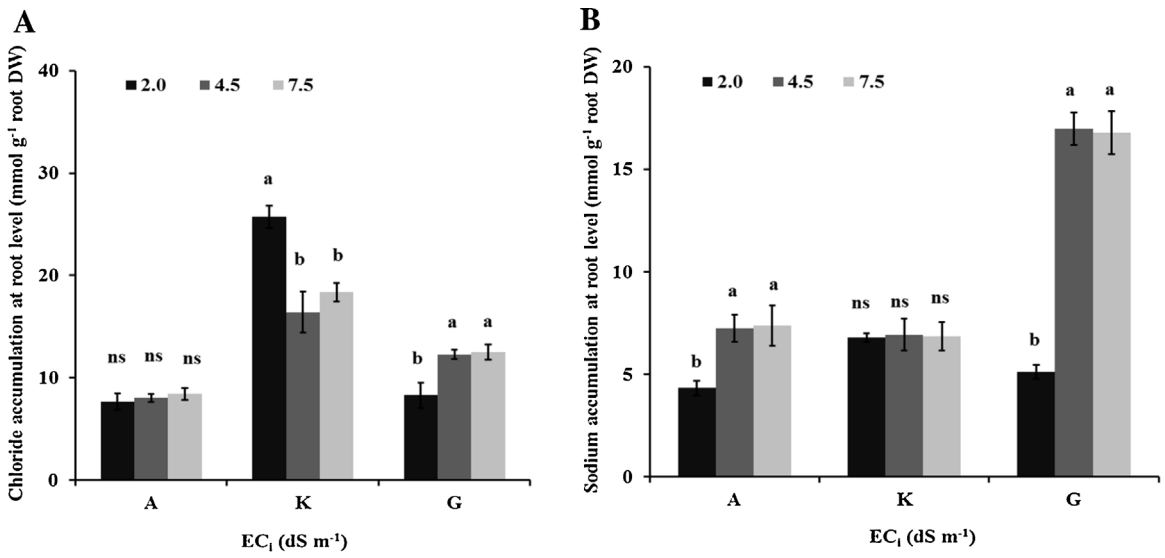

Fig. 2. Effect of EC_i (2.0, 4.5 and 7.5 dS m^{-1}) on chloride (A) and sodium (B) accumulation in roots (Cl^- and Na^+ extraction per plant in mmol/root DW in g) of *A. vera* (A), *K. blossfeldiana* (K) and *G. splendens* (G) at the end of the experiment. Values are the means ± standard deviation (error bars) of four plants per treatment. Means without the same letter in bars are significantly different at $P < 0.05$ (ANOVA and LSD test).

Table 1
Effect of EC_i (2.0, 4.5 and 7.5 dS m^{-1}) on leaf (LDW), root (RDW) and total plant dry weight (TDW) (expressed in gram), leaf weight ratio (LWR), relative root weight ratio (RWR), water content in leaves (WC_l) and roots (WC_r) in *A. vera* (A), *K. blossfeldiana* (K) and *G. splendens* (G) plants at the end of the experiment. Values are the means ± standard deviation of four plants per treatment. Means within a column within a species without the same letter are significantly different at $P < 0.05$ (ANOVA and LSD test).

EC_i (dS m^{-1})		RDW	LDW	TDW	LWR	RWR	WC_l	WC_r
A	2.0	7.73 ± 0.63 a	54.27 ± 3.34 a	64.50 ± 4.34 a	0.82 ± 0.05 a	0.10 ± 0.02 c	0.96 ± 0.01 a	0.87 ± 0.02 a
	4.5	8.39 ± 1.02 a	44.33 ± 3.17 b	55.21 ± 2.59 b	0.80 ± 0.08 a	0.14 ± 0.02 b	0.95 ± 0.01 a	0.87 ± 0.01 a
	7.5	7.99 ± 1.14 a	35.69 ± 3.41 c	45.68 ± 3.19 c	0.82 ± 0.04 a	0.18 ± 0.01 a	0.95 ± 0.01 a	0.85 ± 0.01 a
K	2.0	1.64 ± 0.11 a	7.51 ± 0.62 a	9.15 ± 0.64 a	0.82 ± 0.01 a	0.14 ± 0.01 c	0.94 ± 0.01 a	0.86 ± 0.02 a
	4.5	1.48 ± 0.29 a	6.06 ± 0.42 b	7.53 ± 0.31 b	0.80 ± 0.09 a	0.18 ± 0.02 b	0.94 ± 0.01 a	0.87 ± 0.01 a
	7.5	1.33 ± 0.37 a	5.19 ± 0.39 c	6.90 ± 0.19 c	0.80 ± 0.09 a	0.23 ± 0.01 a	0.92 ± 0.03 a	0.87 ± 0.01 a
G	2.0	2.49 ± 0.47 a	22.72 ± 0.80 a	25.01 ± 0.38 a	0.87 ± 0.05 a	0.09 ± 0.02 c	0.80 ± 0.02 a	0.86 ± 0.01 a
	4.5	2.74 ± 0.51 a	17.99 ± 0.87 b	20.93 ± 0.41 b	0.86 ± 0.02 a	0.14 ± 0.01 b	0.83 ± 0.02 a	0.86 ± 0.01 a
	7.5	2.83 ± 0.43 a	14.46 ± 0.45 c	17.29 ± 0.29 c	0.85 ± 0.04 a	0.17 ± 0.01 a	0.84 ± 0.03 a	0.86 ± 0.02 a

plants in all the species at the end of the experiment. The water content (WC) was unaffected by EC_i irrespective of the analysed organ (roots or leaves) in all species.

3.2. Yield response salinity models

The relative yield (Yr) was related to the electrical conductivity of irrigation water (EC_i) in all species at the end of the experiment through the best models with the largest R^2 value, the Yr being inversely proportional to the EC_i (Fig. 1).

3.3. Physiological measurements

Under different EC_i, the Cl^- and Na^+ root accumulation was different between the species (Fig. 2). The *A. vera* plants remained with no significant differences on Cl^- accumulation between the saline treatments, whereas the Na^+ accumulation increased significantly at higher EC_i comparing to the control at the end of the experiment. Contrastingly, the *K. blossfeldiana* plants irrigated with 4.5 and 7.5 dS m^{-1} decreased the Cl^- accumulation with respect to the control, while no significant effect for the Na^+ accumulation was found 60 days from salinization for all the treatments. In *G. splendens* plants, the Cl^- and Na^+ accumulation at 4.5 and 7.5 dS m^{-1} were greater than those for the control treatment (2.0 dS m^{-1}).

The leaves of *A. vera* and *K. blossfeldiana* did not secrete Cl^- and Na^+ whereas in the *G. splendens* plants, the Cl^- and Na^+ secretion by the leaves increased with increasing salinity treatments (Table 2).

The effects of different EC_i on Cl^- and Na^+ concentration in the shed leaves were different in all the species at the end of the experiment (Fig. 3). *A. vera* did not shed leaves in any treatment.

In shed leaves of *K. blossfeldiana*, Cl^- concentration was similar between treatments, while Na^+ concentration increased significantly with respect to the control treatment. *G. splendens* showed a clear increase of Cl^- and Na^+ concentrations in the shed leaves at greater EC_i (4.5 and 7.5 dS m^{-1}) at the end of the saline period. *K. blossfeldiana* lost 1.5–3.0% of leaves DW in relation to TDW, whereas *G. splendens* sheds leaves in a percentage from 15 to 21% of TDW (data not shown).

The plants showed different trends in leaf succulence index (SI) (Fig. 4). In *K. blossfeldiana* plants, SI remained unchanged across the treatments. The SI of *A. vera* plants decreased significantly at 4.5 and 7.5 dS m^{-1} compared with the control treatment (2.0 dS m^{-1}), whereas in *G. splendens*, SI increased significantly at higher EC_i.

3.4. Ion concentrations in tissues

The mineral composition of the roots and leaves of all the species was affected by the salinity treatments (Table 3). In *A. vera* plants, leaf N decreased with higher EC_i, while there was no clear response in the roots. The root P was similar in all treatments, whereas in the leaves of the plants irrigated with 4.5 dS m^{-1} showed the greatest concentration. The K concentration in the roots and leaves of the plants irrigated with higher EC_i (4.5 and 7.5 dS m^{-1}) were lower than in the control plants.

Table 2
Effect of EC_i (2.0, 4.5 and 7.5 dS m^{-1}) on Cl (A) and Na (B) in washed leaves (WL) and in unwashed leaves (UL). DW: dry weight. For each treatment, Cl^- and Na^+ concentration in WL and UL in the same column with the same letters are not significantly different at $P < 0.05$ (ANOVA and LSD test). For each treatment, the difference between UL and WL in the same row with the same letters are not significantly different at $P < 0.05$ (ANOVA and LSD test). Data are the means ± standard deviation of four plants per treatment.

Species	Element concentration (mmol g^{-1} DW)		EC_i (dS m^{-1})		
			2.0	4.5	7.5
A	Cl^-	WL	1.36 ± 0.03 a	1.38 ± 0.02 a	1.36 ± 0.01 a
		UL	1.40 ± 0.03 a	1.40 ± 0.01 a	1.38 ± 0.02 a
	Cl^-	(UL-WL)	0.03 ± 0.01 a	0.03 ± 0.01 a	0.02 ± 0.01 a
	Na^+	WL	0.81 ± 0.05 a	1.24 ± 0.03 a	1.49 ± 0.05 a
		UL	0.83 ± 0.03 a	1.23 ± 0.05 a	1.50 ± 0.04 a
	Na^+	(UL-WL)	0.01 ± 0.001 a	0.01 ± 0.001 a	0.01 ± 0.001 a
K	Cl^-	WL	1.31 ± 0.06 a	1.45 ± 0.02 a	1.46 ± 0.03 a
		UL	1.35 ± 0.04 a	1.49 ± 0.03 a	1.49 ± 0.04 a
	Cl^-	(UL-WL)	0.03 ± 0.01 a	0.04 ± 0.01 a	0.03 ± 0.01 a
	Na^+	WL	0.30 ± 0.01 a	0.74 ± 0.01 a	0.67 ± 0.01 a
		UL	0.31 ± 0.01 a	0.75 ± 0.01 a	0.69 ± 0.02 a
	Na^+	(UL-WL)	0.01 ± 0.001 a	0.01 ± 0.001 a	0.01 ± 0.002 a
G	Cl^-	WL	1.43 ± 0.02 b	2.51 ± 0.04 b	2.67 ± 0.04 b
		UL	1.62 ± 0.04 a	2.73 ± 0.04 a	2.98 ± 0.05 a
	Cl^-	(UL-WL)	0.18 ± 0.01 c	0.21 ± 0.01 b	0.26 ± 0.01 a
	Na^+	WL	1.19 ± 0.02 b	3.68 ± 0.08 b	4.05 ± 0.09 b
		UL	1.46 ± 0.05 a	4.01 ± 0.06 a	4.53 ± 0.08 a
	Na^+	(UL-WL)	0.27 ± 0.01 c	0.34 ± 0.01 b	0.46 ± 0.02 a

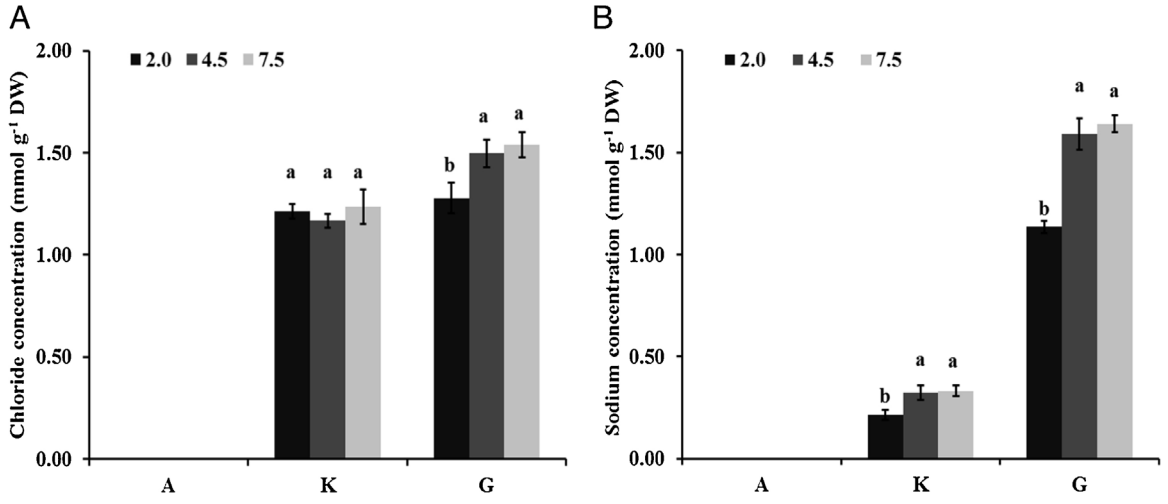

Fig. 3. Effect of EC_i (2.0, 4.5 and 7.5 dS m^{-1}) on chloride (A) and sodium (B) concentration in the shed leaves in *A. vera* (A) *K. blossfeldiana* (K) and *G. splendens* (G) plants at the end of the experiment. Values are the means ± standard deviation (error bars) of four plants per treatment. Means without the same letter in bars are significantly different at $P < 0.05$ (ANOVA and LSD test).

Table 3
Mineral composition (expressed in mmol g^{-1} DW) in roots (R) and leaves (L) of *A. vera* (A), *K. blossfeldiana* (K) and *G. splendens* (G) at the end of the experiment. Values are the means ± standard deviation of four plants per treatment. Means within a column without the same letter are significantly different at $P < 0.05$ (ANOVA and LSD test).

ECi (dS m^{-1})		N		P		K	
		L	R	L	R	L	R
A	2.0	2.02 ± 0.16 a	1.27 ± 0.15 a	0.06 ± 0.01 b	0.10 ± 0.01 a	1.15 ± 0.03 a	0.44 ± 0.01 a
	4.5	0.84 ± 0.23 b	0.81 ± 0.08 b	0.09 ± 0.01 a	0.11 ± 0.01 a	1.09 ± 0.01 b	0.37 ± 0.03 b
	7.5	1.08 ± 0.16 b	1.28 ± 0.08 a	0.03 ± 0.01 c	0.11 ± 0.01 a	1.01 ± 0.03 c	0.32 ± 0.01 b
K	2.0	1.18 ± 0.08 b	1.11 ± 0.09 ab	0.08 ± 0.01 a	0.05 ± 0.01 b	0.97 ± 0.01 a	0.48 ± 0.02 a
	4.5	1.49 ± 0.11 a	1.31 ± 0.11 a	0.08 ± 0.01 a	0.07 ± 0.01 a	0.94 ± 0.04 a	0.31 ± 0.04 b
	7.5	1.43 ± 0.16 a	1.02 ± 0.09 b	0.07 ± 0.01 a	0.03 ± 0.01 c	0.96 ± 0.03 a	0.25 ± 0.03 b
G	2.0	2.03 ± 0.10 a	2.26 ± 0.07 a	0.12 ± 0.01 a	0.10 ± 0.02 b	1.30 ± 0.03 a	0.47 ± 0.04 a
	4.5	1.49 ± 0.07 b	1.20 ± 0.04 c	0.12 ± 0.01 a	0.25 ± 0.01 a	1.19 ± 0.04 b	0.48 ± 0.04 a
	7.5	1.87 ± 0.10 a	1.60 ± 0.07 b	0.04 ± 0.01 b	0.10 ± 0.01 b	1.11 ± 0.02 c	0.45 ± 0.03 a

In control plants of *K. blossfeldiana*, the leaf N level was lower than in the other salt treatments, whilst the root N showed the highest concentration at 4.5 dS m^{-1}. The leaf P was not affected by the salt treatments, while in the roots P was highest in the plants subjected to 4.5 dS m^{-1}. The leaf K was similar in all treatments, whereas the highest value in the roots was observed in the control plants (2.0 dS m^{-1}).

In *G. splendens* plants, leaf N was lowest at 4.5 dS m^{-1}, while in the roots the control plants showed the highest concentration. The plants treated with 7.5 dS m^{-1} showed the lowest leaf P, whereas

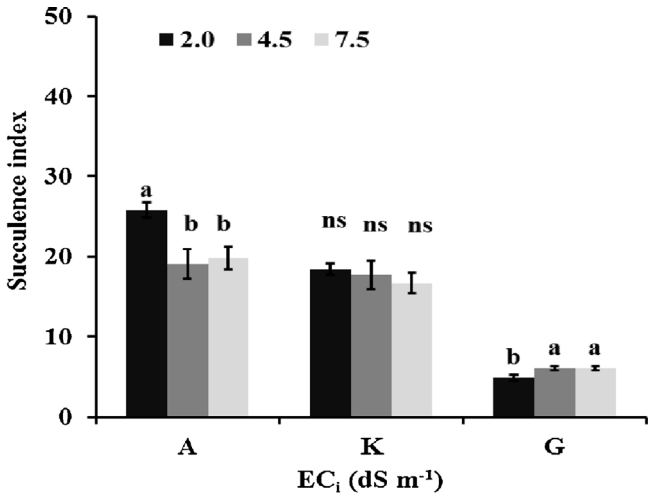

Fig. 4. Effect of EC_i (2.0, 4.5 and 7.5 dS m^{-1}) on succulence index (SI) in leaves of *A. vera* (A), *K. blossfeldiana* (K) and *G. splendens* (G) at the end of the experiment. Values are the means ± standard deviation (error bars) of four plants per treatment. Means without the same letter in bars with the same colour are significantly different at $P < 0.05$ (ANOVA and LSD test).

in the roots, the highest concentration was observed at 4.5 dS m^{-1}. The leaf K decreased significantly with the EC_i increase, whilst in the roots it remained constant in all treatments.

3.5. Biochemical analysis

In *A. vera* plants, the sugar concentration in the roots was higher than in the leaves. The leaf sugar increased significantly with the highest EC_i (7.5 dS m^{-1}), while in the root the highest concentration was found in plants grown in 4.5 dS m^{-1} (Table 4). In the plants treated with 4.5 and 7.5 dS m^{-1}, the proline concentration in the roots and leaves were higher than in the control plants.

In *K. blossfeldiana* plants, the sugar concentration in the roots was lower than in the leaves. Salinity led to a reduction of the sugar concentration in the leaves, whereas in the roots sugar remained unchanged. The proline concentration in the roots and leaves increased with the EC_i and the highest values were observed at 7.5 dS m^{-1}.

In *G. splendens* plants, the sugar concentration in the roots was higher than in the leaves. In the control plants (2.0 dS m^{-1}), the leaf sugar concentration was higher than in the other treatments, while the sugar concentration in the roots showed no differences between treatments. The leaf and root proline concentration increased with the higher EC_i.

4. Discussion

The irrigation water salinity had different effects on the biomass and biochemical parameters according to the species. Under increasing EC_i, the proportion of biomass allocated to leaves (LWR) was not affected in any of the three species tested, thereby allowing the production of marketable plants with moderate salinity. The same results were also observed in *A. vera* plants irrigated with diluted seawater (Jiang et al., 2014), while LWR increased in other potted plants as *Asteriscus maritimus* (Rodríguez et al., 2005) irrigated with 140 mM NaCl and *Cistus albidus* and *C. monspeliensis* (Torrecillas et al., 2003) irrigated with 70 and 140 mM NaCl. After 60 days of exposure, the increase in RWR in the three species we investigated suggests that these potted plants altered the pattern of dry matter distribution favouring root growth over shoot growth as reported by Cordovilla et al. (2014) in *Thymus vulgaris*. In contrast, Jiang et al. (2014) reported a decrease in RWR in *A. vera* plants. Changes in TDW was attributed to LDW and not to RDW. In our experiment, water content was not affected by salinity. These results disagreeing with Jin et al. (2007) and Zheng et al. (2009) who reported a decrease in water content in the different organs of *A. vera* plants assessed with 60% seawater and 200 mM NaCl (higher salinity treatments), respectively; and with the data obtained by Niu and Rodríguez (2006) in other ornamental potted plants as *Penstemon eatonii*, *Delosperma cooperi* and *Gazania rigens* irrigated at higher EC levels (12 dS m^{-1}) which also showed a decline in the water content in the roots and leaves. These apparently contradictory results may be explained by the lower EC_i levels imposed in ours treatments. Although some differences did occur in leaves biomass, they were not expressed in the water content of the tissues. It is also possible that if the experiment had be extended for a longer period (>60 days) water content will probably be affected.

Saline conditions induces an increase in Cl^- and Na^+ concentrations in the roots and leaves in ornamental potted species as reported by Cassaniti et al. (2009). Under these conditions, the tolerance to salinity in each species is triggered through different strategies such as ions accumulation at root level, shedding of dry leaves, salt secretion and succulence in the different organs (Aslam et al., 2011). In our experiment, *A. vera* plants were able to accumulate only Na^+ in roots while *G. splendens* accumulated Cl^- and Na^+ which agrees very well with the conclusion reported by Sykes (1993) explaining that the ability between species to accumulate Cl^- ions in the roots is independent of its ability to accumulate Na^+ ions; a good Cl^- ion excluder is not necessarily a good Na^+ excluder, and vice versa. The shedding of the old leaves with high amounts of Na and Cl, as a mechanism to overcome the salinity proposed by Koyro et al. (2011) was only performed in *K. blossfeldiana* and *G. splendens* plants even though the shedding led a slight reduction in TDW and therefore a lower quality of saleable plants.

A. vera and *K. blossfeldiana* are characterized by the lack of salt glands whereas *G. splendens* has salt glands. A histoanatomical study of leaves in *K. blossfeldiana* (Şipoş and Bunta, 2011) reported the lack of trichomes which explains the absence of salt on the leaf surface and the absence of any differences in the loss of Cl^- and Na^+ by leaves in this species observed in our experiment. Wu et al. (2001) also did not find salt secretion in other ornamental potted plants as *Ceanothus* sp. and *Nandina domestica,* treated with salinity, presumably because of the absence of glands on the leaf surface. Furthermore, in the case of *G. splendens*, the mechanism of Na and Cl excretion by leaves through trichomes is also observed in other plants well adapted to salinity, such as *Atriplex* sp. (Wahid, 2003).

Salt stress alters the succulence of the leaves which is consistent with a vacuolar compartmentation of ions. In our experiment, each species showed a different pattern under increasing NaCl concentration. The decrease of succulence in *A. vera* plants can be interpreted as a mechanism of solute concentration in the cell sap thus contributing to the osmotic adjustment (Matoh et al., 1988). In contrast, it is possible that *G. splendens* had promoted a greater uptake of water in order to dilute the excess of salts (Munns et al., 1983) thus leading to a higher SI in leaves. In the case of *K. blossfeldiana* plants, the succulence index was not affected and this can be due to the lower levels of salinity applied to promote the succulence in this plant.

The excessive accumulation of salts may cause a change on the uptake of mineral nutrients as well as induce phytotoxicity (Hu and Schmidhalter, 2005). The present results showed that the N and P concentrations in the roots and leaves in all the species did not show a clear response to salinity, whereas the K concentration tended to remain without changes or to decrease with the salt stress: *K. blossfeldiana* was the best species maintaining its nutrient status. Contradictory and variable results can be found in the

Table 4
Osmolyte concentration (total soluble sugars (TSS) and proline) expressed in μmol g^{-1} FW in roots (R) and leaves (L) of *A. vera* (A), *K. blossfeldiana* (K) and *G. splendens* (G) plants treated over a period of 60 days with different electrical conductivity of the irrigation water (EC_i). Values are the means ± standard deviation of four plants per treatment. Means within a row without the same letter are significantly different at $P < 0.05$ (ANOVA and LSD test).

			EC_i (dS m^{-1})		
			2.0	4.5	7.5
A	TSS	L	17.11 ± 0.28 b	16.44 ± 0.94 b	35.11 ± 4.38 a
		R	97.22 ± 16.39 ab	115.11 ± 10.88 a	74.39 ± 14.78 b
	Proline	L	0.09 ± 0.01 c	0.19 ± 0.03 b	0.29 ± 0.03 a
		R	0.13 ± 0.01 c	0.16 ± 0.01 b	0.28 ± 0.03 a
K	TSS	L	19.33 ± 0.77 a	11.28 ± 0.89 b	12.06 ± 0.72 b
		R	9.17 ± 1.00 a	8.67 ± 0.88 a	9.83 ± 0.61 a
	Proline	L	0.75 ± 0.10 c	1.15 ± 0.11 b	1.58 ± 0.17 a
		R	0.63 ± 0.04 c	0.77 ± 0.08 b	1.55 ± 0.16 a
G	TSS	L	27.50 ± 0.78 a	18.83 ± 1.17 b	18.67 ± 1.00 b
		R	48.72 ± 2.61 a	50.33 ± 0.83 a	50.94 ± 2.33 a
	Proline	L	2.56 ± 0.19 c	3.35 ± 0.16 b	3.82 ± 0.17 a
		R	1.51 ± 0.50 c	2.47 ± 0.30 b	4.93 ± 0.39 a

literature. Several studies reported that the reduction of the N concentration attributed to the Cl^- and NO_3^- antagonism (Abdelgadir et al., 2005), whereas others reported an increase mainly due to the accumulation of N-containing compounds, such as amino acids including proline in response to salt stress (Parida et al., 2002). On the other hand, the P concentration in plants can show different trends under salinity. Some researchers found a decline due to competition between Cl^- and $H_2PO_4^-$ (Kaya et al., 2001), while others reported a rise due to the energy (ATP) required to transport the excess of ions into the vacuoles (Mengel and Kirkby, 2001). The decrease of K^+ concentration in organs of salinized plants in our experiment indicates the existence of competition effects between Na^+ and K^+ ions which most likely share the same transport system (Blumwald, 2000; Tester and Davenport, 2003; Parida and Das, 2005). In accordance, Cassaniti et al. (2009) reported a decrease on K^+ concentration in other ornamental potted plants as *Bougainvillea glabra*, *Grevillea juniperina* and *Leptospermum scoparium* subjected to salinity.

Osmotic adjustment in plants can be performed through accumulation of osmolytes which are compatible with the cells metabolism (Hasegawa et al., 2000). In our experiment, the changes in roots and leaves in sugar concentrations did not show a defined pattern in response to different NaCl concentrations, except for the decrease in leaves of *K. blossfeldiana* and *G. splendens*. Conversely with these results, other researchers reported an increase of sugar concentration in roots and leaves under salt stress in other ornamental plants as *Melissa officinalis* (Khalid and Cai, 2011). On the other hand, from the results of this experiment, proline is confirmed to be a good stress index for salinity in all the species due to the increment together with the increase in NaCl concentration. The proline concentration increase in the root and leaf in all species under salinity may be the result of a decrease in the proline degradation (Hare et al., 1999) or of an increase in the proline biosynthesis (Lutts et al., 1999). In accordance with our results, Murillo-Amador et al. (2014) reported an increase of the proline concentration in the leaves of *A. vera* plants and other researchers also noted this increase in other ornamental potted plants as *Gerbera jamesonii* L. (Don et al., 2010) and *Delonix regia* (Patel et al., 2009) subjected to the salt stress.

Finally, the results for biomass production, expressed as TDW which is one of the basic requisites for the nursery industry, showed a similar decrease about 30% in all the species under salinty. These values are far from the acceptable percentage of decrease (45%) proposed by local ornamental growers to produce marketable plants, therefore the use of moderate EC_i levels as studied in our trial are feasible for the cultivation of these ornamental species in a controlled environment.

5. Conclusions

Salinity triggered different responses in each species. At the end of the saline period, all species showed a similar decrease on the total biomass and the mineral contents in the roots and leaves were clearly affected. Higher salinity led to an increase in the proline concentration, which may be proposed as a salinity stress indicator, whereas the sugar concentration did not show a clear trend. The different mechanism of salt tolerance evolved in each specie were: (1) *A. vera* plants accumulated Na^+ at root level and decreased the succulence in leaves, (2) *K. blossfeldiana* plants avoided Na^+ accumulation by shedding leaves and (3) *G. splendens* plants triggered Cl^- and Na^+ accumulation at root level, salt secretion through leaves, shedding of old leaves and succulence increase in leaves. These results suggest the importance of studying the salt response of ornamental potted plants to help the growers and gardeners to select the species which are more tolerant to salt stress. *G. splendens* seems to be the most tolerant because evolved more mechanisms of salt tolerance with the same reduction in the biomass than the others species studied.

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