Journal of Applied Botany and Food Quality 95, 17 - 22 (2022), DOI:10.5073/JABFQ.2022.095.003

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Only low saline conditions benefited yield and quality of iceplant grown in pot culture

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(Submitted: November 3, 2021; Accepted: January 10, 2022)

Summary

Mesembryanthemum crystallinum L. or iceplant is an annual facultative halophyte adapted to extreme environmental conditions, as salinity. This characteristic has special importance in the Mediterranean region, where the drought conditions entail the most important problems of soil and water salinity. Furthermore, there is currently a great gastronomic interest for this plant due to its soft texture, fresh and salty taste, high content of water and beneficial compounds for health. Therefore, the objective of this work was to establish the optimal salt growth conditions for pot cultivation of iceplant in greenhouse. Thus, the effect of different salinity levels (0, 100, 200 and 300 mM NaCl) under controlled conditions was evaluated.

Severe salinity treatments reduced crop production. However, results showed that the 100 mM NaCl treatment benefited plant growth. This treatment showed greater leaf fresh weight and area, pigment content and maintained its root and shoot biomass similar to control values. In addition, compared to control plants, salinity increased the specific leaf area and leaf relative water content, reduced leaf starch and K concentrations. Therefore, results confirm that iceplant pot culture can be strategically perform under low salinity conditions with an enhancement or maintenance of crop yield and quality.

Keywords: *Mesembryanthemum crystallinum*, salinity, growth, production.

Introduction

It is known that soil salinity can reduce crop, horticulture and forage production in arid and semiarid regions. Salt may arise naturally in the subsoil or be introduced by brackish irrigation waters. In any case, salinity is becoming more extensive as a result of land clearing and unsustainable irrigation practices and through pressures for bringing marginal land into production (MUNNS and GILLIHAM, 2015). Hence, intensified use of halotolerant crop plants will be necessary, even in Europe.

In this context, commercial use of halophytes as fresh food is nowadays very limited. Several facultative halophytic members of Aizoaceae are used as special crop plants since many years (HERPPICH et al., 2008). Per example, an old, but rare salad green is the common iceplant or glacier lettuce, *Mesembryanthemum crystallinum* (VOGEL, 1996). This species is an annual facultative halophyte with a crassulacean acid metabolism (CAM), which is induced by saline stress, among others. It is an ephemeral or pseudo-biennial prostrate white flowering herbaceous weedy plant with simple, ovate, succulent leaves and stems which are densely covered with bladder cells. These cells give the plant a glistening appearance (ADAMS et al., 1998). It is native from South and East Africa (WINTER, 1978) and is introduced in Western Australia and in the Mediterranean amongs others (WINTER and SMITH, 1996).

With its succulent, mellow, slightly salty tasting leaves and young shoots, this species is a delicious cool flavored salad green. In Europe it is also known as a quickly cooked tender vegetable (HERPPICH et al., 2008). On the other hand, *M. crystallinum* was classified as a highly functional food according to the work of AGARIE et al. (2009), due to high concentration of polyols that is increased under conditions of saline and water stress. The high salt tolerance and content in bioactive compounds and its excellent capacity for phytoremediation, makes the ice plant an important candidate for use as human food and medical care, and in the decontamination of polluted sites (LOCONSOLE et al., 2019).

The effects of environmental stress factors have frequently been studied in *M. crystallinum*, as salt stress (AGARIE et al., 2009; OTSUKA et al., 2021). But more studies are needed to determine the optimum conditions in pot cultivation of *M. crystallinum* to obtain maximum yield and market introduction, to boost the use of this wild edible plant. Therefore, the objective of this work was to establish the non detrimental saline growth conditions for the cultivation of glacier lettuce in greenhouse. To this end, the effect of different irrigation salinity levels on edible leaf production and quality were evaluated.

Materials and methods

Plant material and growth conditions

Seeds of *Mesembryanthemum crystallinum* L. were collected from wild plants of Alicante (SE Spain), disinfected with 0.5% NaClO during 2 h and pre-hydrated with aerated, deionised water for 22 h. Then, they were germinated in vermiculite hydrated with deionised water and mantained in a growth chamber with an air temperature (T) of 24 °C day/night (D/N) and relative humidity (RH) of 70% D/N (THOMAS et al., 1998). Light conditions of the chamber were 16 h light-8 h dark cycle with a photosynthetically active radiation of 400 µmol $m^{-2}s^{-1}$ supplied by a combination of fluorescent tubes (Philips TLD 36W/83 Germany and Silvana F36W/GRO, USA). After 20 days, seedlings were transferred to a greenhouse under semi-controlled conditions of T D/N: 25/18 °C; RH D/N: 60/80% and received natural daylight (mean photosynthetic photon flux rate of 400 µmol m^{-2} s^{-1}) (AGARIE et al., 2007). Then, seedlings were transplanted to 1 L plastic pots with vermiculite watered weekly with 500 ml of Hoagland solution. After 55 days of transplantation, plants were divided into 4 homogeneous groups of 6. Four saline treatments were compared: control (0 mM), 100 mM, 200 mM and 300 mM of NaCl. To avoid an osmotic shock, the concentration of NaCl was increased gradually during the first week to reach the desired NaCl concentration and maintained for one additional week. After 15 days of salinity conditions, plants were harvested and the different determinations were performed. At the end of the experiment, the electrical conductivity (EC) of the substrate from non-saline container and the pots cultivated under 100, 200 and 300 mM of NaCl was 1.80, 13.67, 19.08, 25.01 dS m^{-1}, respectively.

Growth parameters

Plant dry weight (DW) was determined after drying fresh matter at 80 °C in an oven until constant weight. In addition, shoot and root length were measured. Leaf area was measured by the app "Easy

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Leaf Area Free" (Easlon and Bloom, 2014) and specific leaf area (SLA) was calculated as the ratio of the leaf area and the dry weight of leaves.

Water status

The leaf relative water content (RWC) was calculated according to Weatherley's method (1950), using the following formula RWC (%): (FW-DW) / (TW-DW) × 100, being FW: fresh weight, TW: turgid weight, and DW: dry weight of the tissue, respectively. Foliar succulence was measured according to Atzori et al. (2017) by the ratio of the FW of the leaves and the foliar area. Midday water potential (Ψ) of the root was measured by a Scholander pressure chamber (SKPM 1450/40, Skye Instruments Ltd., United Kingdom). All the determinations were performed in expanded young leaves collected at noon.

Biochemical analysis

These analyses were performed on the youngest full-mature leaves harvested at midday, frozen in liquid nitrogen and stored at -20 °C for later quantifications. The concentration of foliar photosynthetic pigments was determined according to Sesták et al. (1971). The samples (20 mg FW) were included in 5 ml of 96% ethanol at 80 °C for 10 minutes to extract the pigments. The absorbance of the extracts was spectrophotometrically measured and the equations reported by Lichtenthaler (1987) were used to calculate the concentration of chlorophylls and carotenoids.

Starch, total soluble sugars (TSS) and proline in leaves were quantified in potassium phosphate buffer (KPB) (50 mM, pH = 7.5) extracts of fresh tissue (0.1 g). These extracts were filtered through four layers of cheesecloth and centrifuged at 28710 g for 15 min at 4 °C. The pellet was used for starch determinations (Jarvis and Walker 1993). The supernatant was collected and stored at 4 °C for TSS and proline determinations. Total soluble sugars were analysed spectrophotometrically with the anthrone reagent (Yemm and Willis, 1954). Free proline was estimated by spectrophotometric analysis at 515 nm of the ninhydrine reaction (Irigoyen et al., 1992).

Mineral analysis

Leaf samples (0.5 g DW) were dry-ashed and dissolved in HCl according to Duque (1971). Magnesium, potassium, phosphorus, calcium, sodium, manganese, zinc and iron concentrations were determined using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, USA). The operating parameters of the ICP-OES were: radio frequency power 1300 W, nebulizer flow 0.85 L min^{-1}, nebulizer pressure 30 psi, auxiliary gas flow 0.2 L min^{-1}, sample introduction 1 ml min^{-1} and three replicates per sample. Total carbon and nitrogen were quantified after combustion (950 °C) of leaf DW with pure oxygen y an elemental analyzer provided with a thermal conductivity detector (TruSpec CN, Leco, USA).

Statistics

The results were analyzed with one-way analysis of variance (ANOVA) by the statistical program SPSS v.26 (IBM Corp., USA). The means ± standard deviation (SD) were calculated and, when the *F* ratio was significant ($p<0.05$), least significant differences were evaluated by the Duncan test. Significance levels were set at 5%.

Results

Growth parameters showed significant differences regarding to the salt treatment (Tab. 1). Plants grown with 100 mM of NaCl maintained high shoot and root biomass, if we compare with the rest of saline treatments. In addition, iceplant subjected to 100 mM of NaCl enhanced significantly leaf area (Tab. 1) and leaf fresh weight (Fig. 1). Values of SLA also increased in plants cultivated with salinity conditions comparing with control ones (Tab. 1). The results of shoot height and root length showed that there were no differences between salinity levels.

With reference to leaf water status, plants grown under salinity over 100 mM NaCl had greater RWC than control plants (Tab. 2). However, the highest leaf succulence was observed in control plants. In relation to root water potential, this parameter did not show a clear trend, with higher values in 100 and 300 mM NaCl treatments in comparison with controls.

Biochemical analysis (Tab. 3) performed on the leaves of iceplant displayed differences between treatments. In relation to the photosynthetic pigments, plants cultivated with 100 mM NaCl increased levels of both chlorophylls and carotenoids concentration. Moreover, although amounts of starch declined in leaves of iceplant due to salt stress, TSS concentration only reduced in treatments of 100 and 300 mM of NaCl. Levels of proline increased in plants subjected to saline conditions over 100 mM.

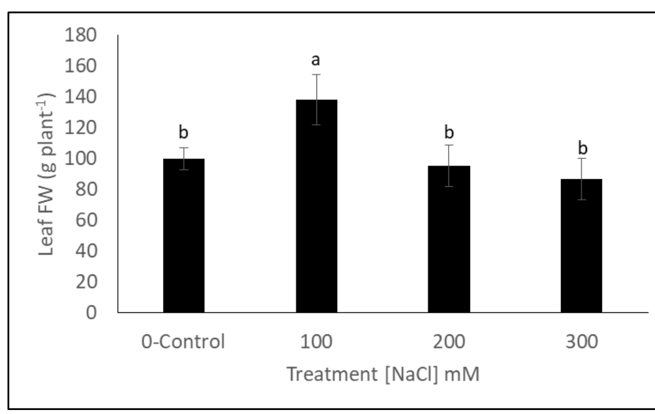

Fig. 1: Leaf fresh weight (FW) in iceplant subjected to different salt conditions. Means (n=6) ± SD were compared with Duncan test. Bars followed by a common letter are not significantly different ($p\leq0.05$).

Tab. 1: Growth parameters in iceplant subjected to different salt conditions.

Treatment [NaCl] mM	Shoot DW (g $plant^{-1}$)	Leaf DW (g $plant^{-1}$)	Root DW (g $plant^{-1}$)	Leaf area (cm^2)	SLA (cm^2 g^{-1})	Shoot height (cm)	Root length (cm)
0-Control	1.95 ± 0.40 ab	1.38 ± 0.29 a	0.91 ± 0.20 ab	139.12 ± 20.00 c	96.47 ± 12.95 b	15.87 ± 2.84 a	38.20 ± 6.14 a
100	2.12 ± 0.35 a	1.45 ± 0.41 a	1.08 ± 0.32 a	243.34 ± 16.47 a	178.97 ± 53.94 a	15.48 ± 3.79 a	35.00 ± 2.24 a
200	1.60 ± 0.19 bc	1.22 ± 0.16 a	0.64 ± 0.11 b	172.63 ± 18.38 b	146.43 ± 15.72 a	16.00 ± 3.24 a	34.58 ± 8.38 a
300	1.46 ± 0.27 c	1.15 ± 0.31 a	0.66 ± 0.21 b	183.10 ± 32.22 b	163.20 ± 28.15 a	15.75 ± 3.42 a	32.25 ± 8.64 a

Means (n=6) ± SD were compared with Duncan test. Within each column, values followed by a common letter are not significantly different ($p\leq0.05$). DW: dry weight.

Tab. 2: Relative water content (RWC), succulence and root water potential (Ψ) in iceplant subjected to different salt conditions.

Treatment [NaCl] mM	RWC (%)	Succulence (g FW cm^{-2})	Root ψ (MPa)
0-Control	88.59 ± 5.39 b	0.73 ± 0.15 a	-0.27 ± 0.08 b
100	94.22 ± 8.59 ab	0.57 ± 0.06 b	-0.10 ± 0.01 a
200	97.67 ± 2.38 a	0.53 ± 0.07 b	-0.25 ± 0.05 b
300	99.12 ± 1.73 a	0.48 ± 0.04 b	-0.15 ± 0.08 a

Means (n=6) ± SD were compared with Duncan test. Within each column, values followed by a common letter are not significantly different (p≤0.05). FW: fresh weight.

The effect of salinity in leaf mineral concentration of iceplant was also assessed (Tab. 4 and 5). Despite leaf concentration of C and N was similar between treatments, plants grown with 300 mM NaCl had significantly greater concentrations of Mg and Ca compared to the rest of treatments (Tab. 4). On the other hand, the amount of K was lower as salt concentration increased over 100 mM and there were no differences between treatments in the amount of P. With respect to the micronutrients (Tab. 5), Mn concentration had not a clear trend related to the effect of salinity and there were no differences between treatments in the amount of Zn. Iceplant that received 100 and 200 mM NaCl showed the highest values of Fe, and as expected, the accumulation of Na was higher as the severity of salt stress increased.

Discussion

High salinity is a critical problem in crop production that results in reduced plant growth and a significant reduction in productivity (Tsukagoshi et al., 2015). *Mesembryanthemum crystallinum* (iceplant) is an halophyte that can survive in high salinity soils and switches from C3 photosynthesis to CAM under high salinity and drought stress (Adams et al., 1998). We have observed that irrigation with 100 mM of NaCl produced an improvement in growth, with important effect on parameters affecting edible parts of the crop, such as the leaf area or leaf fresh weight. In addition, the application of higher concentration of salt generated growth reduction. These results are in accordance with the study of Winter (1973), in which the maximum growth of iceplant was obtained with a concentration of 100 mM NaCl. Rodríguez-Hernández and Garmendia (2021) showed that a moderate concentration of salinity (100 mM NaCl) improved the accumulation of nutraceuticals and the production of edible leaves while high saline conditions were detrimental to the plant's growth. Atzori et al. (2017) also described the increase of leaf biomass and area in plants subjected to moderate salinity. In this regard, it should be noted that most halophytes as iceplant require saline conditions to attain optimal growth. According to Flowers et al. (1986) *M. crystallinum* shows optimal growth within 50-250 mM NaCl. Thus, in this study it was confirmed that *M. crystallinum* irrigated with 100 mM NaCl for two weeks had the highest leaf FW and leaf area. Similarly, He et al. (2021) recently showed that 100 mM NaCl had the highest shoot FW and largest leaf area compared with those grown at 0 and 250 mM NaCl for 15 days. This

Tab. 3: Leaf photosynthetic pigment concentration and leaf concentration of starch, total soluble sugars (TSS) and proline in iceplant subjected to different salt conditions.

Treatment [NaCl] mM	Chlorophylls (mg g^{-1} DW)	Carotenoids (mg g^{-1} DW)	Starch (mg g^{-1} DW)	TSS (mg g^{-1} DW)	Proline (mmol g^{-1} DW)
0-Control	32.30 ± 7.49 b	5.35 ± 1.10 ab	309.01 ± 90.18 a	189.85 ± 52.17 a	43.66 ± 12.77 c
100	41.60 ± 7.20 a	6.47 ± 1.76 a	84.83 ± 34.52 b	119.70 ± 27.76 b	41.39 ± 11.72 c
200	29.79 ± 3.56 b	4.22 ± 0.64 bc	37.53 ± 13.05 bc	193.60 ± 56.79 a	101.37 ± 30.41 b
300	29.27 ± 7.94 b	3.17 ± 0.88 c	20.06 ± 9.80 c	94.40 ± 25.99 b	203.87 ± 47.56 a

Means (n=6) ± SD were compared with Duncan test. Within each column, values followed by a common letter are not significantly different (p≤0.05). DW: dry weight.

Tab. 4: Foliar concentrations of macronutrients in iceplant subjected to different salt conditions.

Treatment [NaCl] mM	C (mg g^{-1} DW)	N (mg g^{-1} DW)	Mg (mg g^{-1} DW)	K (mg g^{-1} DW)	P (mg g^{-1} DW)	Ca (mg g^{-1} DW)
0-Control	431.17 ± 119.95 a	56.39 ± 15.78 a	18.49 ± 3.49 b	43.78 ± 6.29 a	2.70 ± 0.83 a	7.55 ± 1.11 b
100	370.33 ± 57.40 a	45.19 ± 7.31 a	17.98 ± 3.60 b	31.82 ± 4.66 b	2.55 ± 0.66 a	7.98 ± 1.78 b
200	395.40 ± 46.25 a	50.29 ± 4.97 a	22.62 ± 4.86 b	30.69 ± 5.55 b	2.35 ± 0.35 a	8.71 ± 2.24 b
300	362.67 ± 40.93 a	46.41 ± 3.55 a	28.16 ± 3.31 a	33.87 ± 6.80 b	2.45 ± 0.49 a	16.02 ± 2.62 a

Means (n=6) ± SD were compared with Duncan test. Within each column, values followed by a common letter are not significantly different (p≤0.05). DW: dry weight.

Tab. 5: Foliar concentrations of micronutrients and sodium in iceplant subjected to different salt conditions.

Treatment [NaCl] mM	Mn (µg g^{-1} DW)	Zn (µg g^{-1} DW)	Fe (µg g^{-1} DW)	Na (mg g^{-1} DW)
0-Control	483.07 ± 66.04 a	67.06 ± 1.79 a	1666.48 ± 474.77 b	3.41 ± 1.02 c
100	320.47 ± 37.30 b	65.60 ± 5.52 a	2305.05 ± 494.23 a	24.01 ± 7.72 b
200	432.41 ± 63.97 a	70.92 ± 7.34 a	1976.63 ± 454.53 a	30.63 ± 4.38 ab
300	349.84 ± 39.88 b	70.54 ± 8.64 a	900.32 ± 375.47 c	37.18 ± 11.07 a

Means (n=6) ± SD were compared with Duncan test. Within each column, values followed by a common letter are not significantly different (p≤0.05). DW: dry weight.

good performance of the iceplant under low salinity conditions, indicates its potential for saline agriculture.

Furthermore, an increase in SLA was observed in our plants subjected to saline stress related to the enhancement of leaf area. It can be suggested that the ice plant leaf area was not reduced by salinity because another feature helped in regulating the leaf ion concentration: the epidermal bladder cells, which function as peripheral salt and water reservoirs (LUTTGE et al., 1978; AGARIE et al., 2007). In addition, it is worth mentioning that the saline treatment applied lasted two weeks. In this short term effect of salinity, concentrations over 100 mM NaCl resulted to be detrimental for iceplant growth under pot culture. According to AGARIE et al. (2007), iceplant subjected to 100 mM NaCl for 3 weeks gained a higher dry matter than the same treatment for a week and even at 2 weeks. Moreover, only much higher saline treatments (800 mM) showed a decrease in dry biomass as the exposure time increased to 3 weeks. Therefore, a longer term treatment of salt stress in iceplant pot culture seems to led to similar results.

The increase in RWC of plants irrigated with NaCl can be explained by the adaptive mechanisms to the saline stress that *M. crystallinum* performs, since the bladder cells accumulate water, in addition to salts, to improve ionic and osmotic stress (AGARIE et al., 2007) and the induction of CAM metabolism causes the plant to lose less water related to stomatal closure during the day. Due to these adaptations it was expected that the iceplant had greater succulence in salinity treated plants. However, since succulence is calculated as the ratio of fresh leaf weight to the leaf area, the maintenance of high leaf area in saline conditions would explain the decrease in succulence according to our data. On the other hand, the expected response of the root water potential was to decrease with the increase of salinity levels, but our results differed. Root Ψ in controls was lower than in the 100 and 300 mM NaCl treatments. Because *M. crystallinum* is a crass plant, the manipulation and determination of Ψ could have hindered using the pressure chamber, which would indicate a possible inaccuracy of these measurements (HERPPICH and HERPPICH, 1997; BUSSO, 2008).

In relation to photosynthetic pigments, total leaf chlorophyll concentration was higher in the plants treated with 100 mM NaCl. According to HERPPICH et al. (2008), the increase in chlorophyll can be explained by a general physiological stimulation in response to salinity. Furthermore, the similar values of chlorophylls in treatments with high salinity levels in relation to the control indicated that in the halophyte *M. crystallinum*, salinity did not destroy chloroplasts or increase the activity of the chlorophyllase enzyme, which affects the synthesis of chlorophylls (ARGENTEL et al., 2006). The concentration of carotenoids was maintained as controls in the treatment of 100 mM NaCl, with significant lower values as saline conditions increased. These results did not coincide with those obtained by ATZORI et al. (2017), where the concentration of carotenoids increased with the increase in salinity. However, REDONDO-GÓMEZ et al. (2010) in *Arthrocnemum macrostachyum* halophilic plant, described a progressive decrease in the concentration of carotenoids in salinity treatments above 100 mM. Carotenoids have a membrane stabilizing role (MORGAN, 2004) and are related to the VAZ or xanthophyll cycle. This cycle dissipates energy in the form of heat, protects chloroplasts against photoxidation caused, for example, by saline stress. Therefore, similar or increased concentration of photosynthetic pigments in plants subjected to a low salt stress of 100 mM comparing with controls, could presume an increase of the photosynthetic capacity together with greater amount of energy in thermal dissipation over photochemistry (ATZORI et al., 2017).

Salinity did not influence negativelly the leaf concentration of C, N, P and Zn. As expected, salinity treatments increased the foliar concentration of Na in iceplant, demonstrating that *M. crystallinum* acts as a sequestrant of salts, due to the presence of bladder cells (AGARIE et al., 2007). Ca and Mg increased with salinity, reaching the maximum concentration of these minerals with 300 mM NaCl. ATZORI et al. (2017) studied the effect of different levels of salinity on *Mesembryanthemum* and found that none of the salinity treatments negatively affected the biomass production of glacier lettuce and Ca leaf concentration increased with increasing salinity. Furthermore, the increase in salinity lengthened the vegetative stage, obtaining an additional month of harvest compared to the non-saline treatment. The significant increase of Ca and Mg concentrations may represent an interesting nutritional improvement achievable in salinity conditions. Calcium is among the main mineral elements lacking in the diet of over two-thirds of the world's population (WHITE and BROADLEY, 2009). On the other hand, Ca and Mg are antagonists of Na, so in plants not adapted to salinity a decrease in Ca or Mg concentrations would be expected, due to a displacement by Na from the cell membrane binding sites (DODD et al., 2010). But, according to BRESSAN et al. (1998), in iceplant, the increase in Ca, as Mg, is a cause of adaptation to salinity, since it has a protective effect on membranes under salinity conditions. In relation to the concentration of K, it decreased with salinity, because Na competes by the binding sites in the transport system that mediates the taking of K (NIU et al., 1995). Our results are in the same line of the study by ATZORI et al. (2017), where Na was higher in iceplant irrigated with salt than in the controls, as well as Ca and Mg, while K decreased with increasing salinity.

Additionaly, starch leaf concentration of glacier lettuce decreased as salinity increased. Similarly, ADAMS et al. (1992) and PARAMONOVA et al. (2004), observed the diminishing of starch content in iceplant grown with NaCl, probably caused by starch degradation under stress conditions. Also, the levels of TSS were lower in salt treatments than in control, in accordance with ATZORI et al. (2017). Nevertheless, plants irrigated with 200 mM presented similar values of sugars as the control treatment, while the starch concentration was lower. On the other hand, proline is part of the osmolytes that act in the osmotic adjustment (HUANG et al., 2000; ZOBAYED et al., 2007). Our results showed that proline increased with the amount of salinity in order to retain water in the cells (CVIKROVÁ et al., 2013), that probably allowed the increase of RWC in plants subjected to saline conditions.

Conclusions

The analysed growth parameters showed that iceplant is a crop suitable for irrigation with saline water, wich is important in Mediterranean region. Thus, the implementation of the pot cultivation of *M. crystallinum* would represent an alternative to the current problem of salinization of arable land and irrigation water, since most traditional crops are sensitive to salinity. Furthermore, in this work it has been described that iceplant irrigated with 100 mM NaCl during 2 weeks increased important growth parameters as leaf area and its fresh weight. These parameters are especially important in crop yield. Also, this treatment of salinity turned out to be the only one capable of maintaining both the shoot and root biomass at values similar to those of the control, while higher salinity conditions were detrimental to growth. On the other hand, aspects highly valued by the consumer in this plant, such as the relative water content and its salty flavor, were enhanced with salinity. Despite the halophilic nature of iceplant, salinity treatments for two weeks over 100 mM were not very suitable for edible fresh leaf production under pot culture.

Acknowledgements

The authors wish to thank to Susana Carrión and Mar Benavent for their help with biomass analysis and laboratory determinations.

Conflicts of interest

No potential conflict of interest was reported by the authors.

Funding
This work was financed by the Valencian Community – Spain (GV/2018/068).

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