

## Age of plant influences the effect of salinity in yield and mineral content of ice plant

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### Summary

The use of salinity-tolerant plants represents a response to the problem of the expansion of salinized soils, making coastal and salt-affected areas productive. Furthermore, limited fresh water resources may increasingly constrain the use of low-quality irrigation water. Therefore, intensified use of halotolerant crop plants will be necessary. *Mesembryanthemum crystallinum* is a salinity-tolerant plant widely distributed and currently with a great gastronomic interest because is considered a functional food. The objective of this work was to evaluate the differential effect of a moderate salinity treatment imposed in ice plants of 40 or 55 days after transplanting. Thus, *M. crystallinum* of 40 and 55 days were grown under 0 and 100 mM NaCl during two weeks. The results showed that the effect of salinity depended of the age of plants. Growth parameters as shoot biomass or shoot height decreased in plants of 40 days after transplanting (DAT) subjected to salinity while no differences were found in 55 DAT plants. Also, salinity improved important yield parameters as leaf fresh mass and area when the treatment was applied in 55 DAT plants and caused higher SLA and chlorophylls content in both groups of plants. Ice plant can be intentionally cultivated 55 DAT under moderate salinity conditions to enhance crop yield which could contribute to a more extensive use of its edible leaves as functional and alternative food.

**Keywords:** *Mesembryanthemum crystallinum*, plant age, glacier lettuce, saline conditions, plant production.

### Introduction

Recent models of global change have predicted a dramatic increase in desertification (SCHNEIDER et al., 2007), saline and arid conditions, and a rise in sea levels, therefore, arable land will be increasingly affected (ATZORI et al., 2017). Furthermore, the need for irrigation will be unavoidable in these locations, limiting freshwater resources and the use of quality irrigation water (KUNDZEWICZ et al., 2007). On the other hand, it must be taken into account that most conventional crops are sensitive to salt (KOYRO et al., 2011), resulting in yield reductions due to water deficit stress, ionic toxicity, nutritional disorders and genotoxicity (MUNNS, 2002). Therefore, the use of salinity-tolerant crops represents a response to the problem of decreased freshwater availability and the expansion of salinized soils, making coastal and salt-affected areas productive (ATZORI et al., 2017). In this way, greater sustainable food production would be achieved. Halophytic species, tolerant to salinity and native to marshes and saline sites, have the characteristic of being able to grow and reproduce in saline soils, where approximately 99% of other species could not (FLOWERS and COLMER, 2008).

An alternative is to implement the cultivation of halophytic or salinity-tolerant plants such as *Mesembryanthemum crystallinum* or ice plant. *Mesembryanthemum crystallinum* is a succulent plant, which has been commonly studied for displaying C3 and CAM photosynthesis in response to environmental stresses such as high salinity

(ABD EL-GAWAD and SHEHATA, 2014). It is native to the Nambian desert in southern Africa and is widely distributed and naturalized in western Australia, southwestern US, the Pacific coast of Mexico and Chile (BOHNERT and CUSHMAN, 2000). During the 18<sup>th</sup> and 19<sup>th</sup> centuries *M. crystallinum* was used in medicine, in bladder problems, gallbladder, whooping cough, tuberculosis or urinary problems, as it acted as a diuretic (RAAK et al., 2014). In this sense, it is already being consumed as a vegetable crop in several countries such as India, California, Australia and New Zealand and in some countries of Europe (AGARIE et al., 2009), i.e. in Germany (HERPPICH et al., 2008) and in The Netherlands (ATZORI et al., 2017). On the other hand, is well known for its antioxidant activity (IBDAH et al., 2002; AGARIE et al., 2009) and the ability to rapidly accumulate phytochemicals and secondary metabolites, such as beta-carotene, in a cell-specific manner (WEEPLIAN et al., 2018). Furthermore, ice plant was classified as a highly functional food due to high concentration of polyols. Previous work carried out by our group have demonstrated that ice plant cultivated under different salt levels respond in a different way. Thus, high level of salinity improved the concentration of bioactive compounds, including its antioxidant properties, but this was in detriment of plant growth. However, irrigation with 100 mM of NaCl improved both the production of edible leaves and the accumulation of nutraceuticals (RODRÍGUEZ-HERNÁNDEZ and GARMENDIA, 2022a). In this sense, controlled deficit irrigation has been proposed as an efficient strategy in terms of agronomic management to reduce the use of this scarce resource and improve the organoleptic and functional quality of tomato (MARTÍ-RENAU et al., 2018). Similarly, it would be interesting to analyze how the timing of salinity conditions affects plant growth and quality. Therefore, we sought to study if the plant age influences the effect of salinity in ice plant. Thus, the objective of this work was to evaluate the distinguishing effect of a moderate salinity level on *M. crystallinum* depending on plant age, focusing on the yield and nutritional quality of its edible leaves.

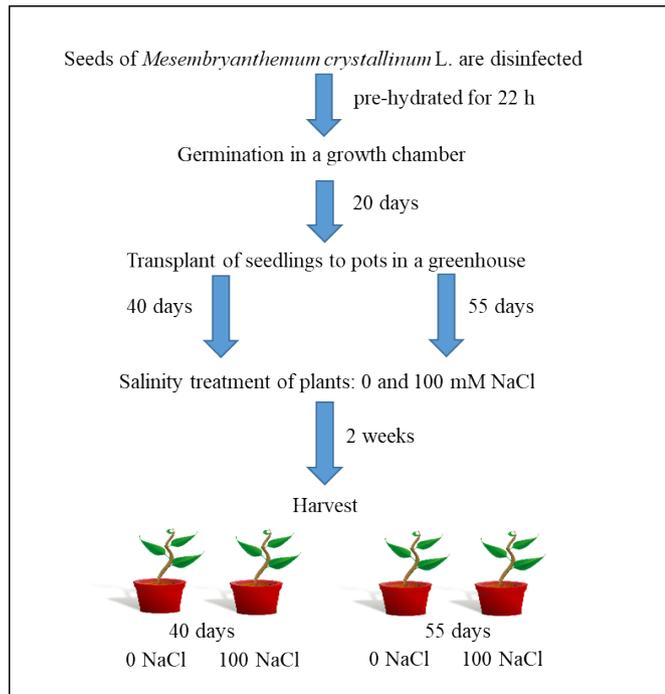
### Materials and methods

#### Plant material and growth conditions

Seeds of *Mesembryanthemum crystallinum* L. were collected from wild plants in Alicante (SE Spain), disinfected with 0.5% NaClO during 2 h and pre-hydrated with aerated, deionized water for 22 h. Seeds were germinated in vermiculite hydrated with deionized water and maintained in a growth chamber set to 24 °C air temperature (T) and 70% relative air humidity (RH), day and night (D/N) (THOMAS et al., 1998). Photosynthetically active photon flux rate in the chamber was approx. 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (at a 16/8 h light-dark cycle) supplied by a combination of fluorescent tubes (Philips TLD 36W/83 Germany and Silvana F36W/GRO, USA). After 20 days, seedlings were transplanted to 1 L plastic pots and 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  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) (AGARIE et al., 2007). Pots with vermiculite were watered twice a week with a total of 300 mL of Hoagland solution. After 40 days, plants were grown under 0 and 100 mM NaCl. Then irrigation with Hoagland solution was increased to

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500 mL per week (divided into two irrigation times) in the whole of plants to supply increasing watering demand. And after more 15 days (55 days after transplanting (DAT)) another group of plants were grown under the same two conditions described previously. To avoid an osmotic shock, the concentration of NaCl was increased gradually during the first week of the salinity treatment to reach the desired NaCl concentration, and maintained for one additional week. After two weeks of salinity conditions, plants were harvested and the different determinations were performed (Fig. 1).



**Fig. 1:** Illustrative diagram of the experimental design.

### Growth parameters

Plant dry mass (DM) was determined after drying fresh matter at 80 °C in an oven until constant mass which was reached after minimum one week. In addition, shoot and root length were measured.

### Leaf production and water status

Production of edible part of the plant was measured as leaf fresh mass (FM), and leaf area which was estimated by the app “Easy Leaf Area Free” (EASLON and BLOOM, 2014). Specific leaf area (SLA) was calculated as the ratio of the leaf area and the dry mass of leaves.

The leaf relative water content (RWC) was recorded according to Weatherley’s method (1950), using the following formula  $RWC (\%) = (FM - DM) / (TM - DM) \times 100$ , being FM: fresh mass, TM: turgid mass, and DM: dry mass of the tissue, respectively. Foliar succulence was measured according to ATZORI et al. (2017) by the ratio of the FM of the leaves and the foliar area.

### Biochemical and mineral analysis

The 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 FM) were included in 5 mL of 96% ethanol at 80 °C for 10 min 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.

For mineral analysis, leaf samples (0.5 g DM) 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<sup>-1</sup>, nebulizer pressure 206.84 kPa, auxiliary gas flow 0.2 L min<sup>-1</sup>, sample introduction 1 mL min<sup>-1</sup> and three replicates per sample. Total carbon and nitrogen were quantified after combustion (950 °C) of leaf DM with pure oxygen and an elemental analyzer provided with a thermal conductivity detector (TruSpec CN, Leco, USA).

### Statistics

The results were analyzed with two-way analysis of variance (ANOVA) by the statistical program SPSS v.26 (IBM Corp., USA). The variance was related to the main factors (salinity treatment and age of plants when salinity was imposed) and to the interaction between them. 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 always set at 5%.

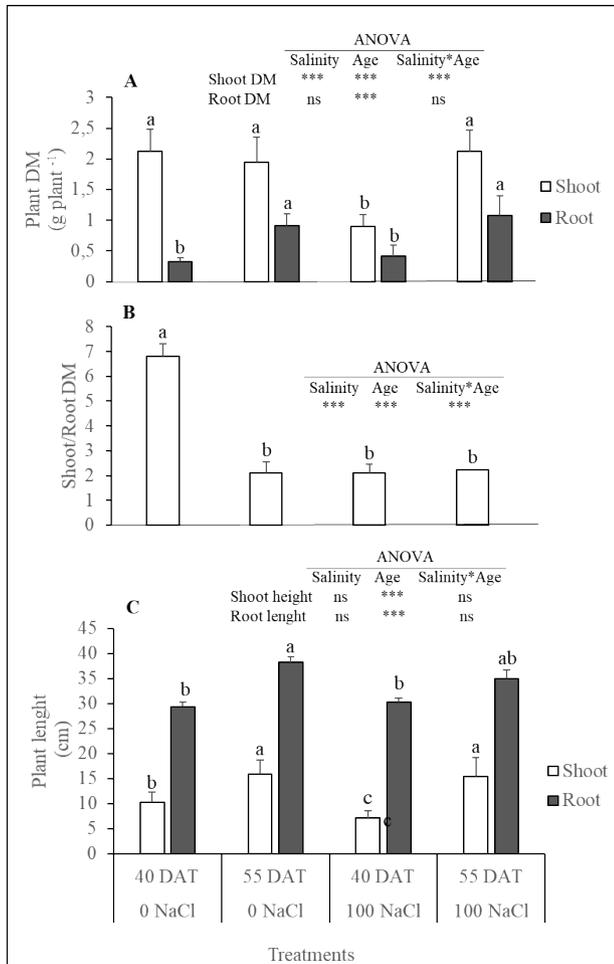
### Results

Regarding to the growth parameters, differences were observed in the analyzed variables (Fig. 2). Salinity did not influence shoot DM when salt condition was imposed 55 days after transplanting, while it reduced shoot growth in 40 DAT plants (Salinity \* Age,  $p < 0.001$ ). Otherwise, salinity did not influence root DM or its length independently of the age of plants (Salinity,  $p > 0.05$ ). However, both parameters were lower in 40 DAT plants (Age,  $p < 0.001$ ). The effect of salinity on shoot to root ratio depended of the age of plants (Salinity \* Age,  $p < 0.001$ ). In fact, it only decreased in plants treated with 100 mM NaCl at 40 DAT. Furthermore, 55 DAT plants had lower shoot to root relation than 40 DAT control plants (Age,  $p < 0.001$ ). Concerning shoot height of the plants, it was significantly lower in 40 DAT treatment subjected to salinity.

The results in Tab. 1 indicated that salinity increased the leaf FM production in 55 DAT plants (Salinity \* Age,  $p < 0.001$ ), while it did not influence in 40 DAT *Mesembryanthemum* plants. As expected, 40 DAT plants had a lower leaf FM than the 55 DAT plants (Age,  $p < 0.001$ ). Regarding to the leaf area and SLA, salinity influenced both parameters (Salinity,  $p < 0.001$ ), with the highest values being found in the saline treatment with 55 DAT plants. On the other hand, leaf RWC in 40 DAT salt-treated plants was lower than that of controls of the same age and both treatments in 55 DAT plants (Age,  $p < 0.05$ ). And leaf succulence was significantly higher in 55 DAT controls than in salt-grown plants of the same age (Salinity \* Age,  $p < 0.01$ ).

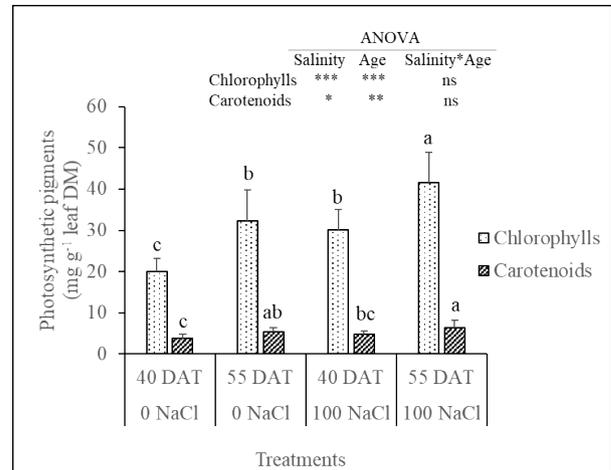
In relation to photosynthetic pigments (Fig. 3), both the concentration of chlorophylls and carotenoids was higher in the saline treatments, especially in 55 DAT plants (Salinity,  $p < 0.05$ ; Age,  $p < 0.01$ ).

Macronutrients (Tab. 2) and micronutrients (Tab. 3) showed, in general, significant differences between treatments. Specifically, although the main factors studied did not have significant effect on the foliar C concentration, the amount of foliar N was reduced due to salt conditions (Salinity,  $p < 0.01$ ). The concentration of P was not influenced by salinity, although it was affected by the age of the plant (Age,  $p < 0.001$ ), with the lowest values presented in 55 DAT plants. Something similar happened with the foliar K content, which presented the lowest values in the saline treatment of 55 DAT plants and did



**Fig. 2:** Shoot and root dry mass (A), shoot to root ratio (B) and shoot and root length (C) in ice plant grown under non-saline (0 mM NaCl) and saline (100 mM NaCl) conditions, 40 or 55 days after transplanting.

Means ( $n=6$ )  $\pm$  SD were compared with Duncan test. Within each parameter, values followed by a common letter are not significantly different ( $p \leq 0.05$ ). Within each graph the explanations for the symbols of ANOVA are: not significant at  $P > 0.05$  (ns), significant at  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*). DAT: days after transplanting. DM: dry mass.



**Fig. 3:** Leaf photosynthetic pigment concentration in ice plant grown under non-saline (0 mM NaCl) and saline (100 mM NaCl) conditions, 40 or 55 days after transplanting.

Means ( $n=6$ )  $\pm$  SD were compared with Duncan test. Within each variable, values followed by a common letter are not significantly different ( $p \leq 0.05$ ). Within the graph the explanation for the symbols of ANOVA are: not significant at  $P > 0.05$  (ns), significant at  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*). DAT: days after transplanting. DM: dry mass.

not show differences between treatments in 40 DAT plants (Salinity \* Age,  $p < 0.05$ ). The content of Ca and Mg was similar in 55 DAT treatments and the 40 DAT plants presented a lower concentration of these nutrients than 55 DAT plants (Age,  $p < 0.001$ ). A very marked reduction of Ca was observed in 40 DAT plants grown with salinity (Salinity \* Age,  $p < 0.01$ ; Salinity,  $p < 0.05$ ) and without significant differences between the saline treatments in the case of Mg (Salinity,  $p > 0.05$ ). The concentration of Mn (Tab. 3) did not show a clearly established pattern, since the salinity reduced its concentration in 55 DAT plants, while its concentration was increased in 40 DAT ones (Salinity \* Age,  $p < 0.001$ ). The opposite occurred in concentration of Fe, with a significant interaction between the main factors studied (Salinity \* Age,  $p < 0.001$ ). In relation to the Zn concentration, no significant differences were observed between treatments in 55 DAT plants while in 40 DAT plants, there was a decrease of the Zn concentration with the salinity. As expected, salinity increased the foliar Na concentration in both groups of plants (Salinity,  $p < 0.001$ ,

**Tab. 1:** Leaf fresh weight (FM), leaf area, specific leaf area (SLA), relative water content (RWC) and succulence in ice plants grown under non-saline (0 mM NaCl) and saline (100 mM NaCl) conditions, 40 or 55 days after transplanting.

NaCl (mM)	Age (DAT)	Leaf FM (g)	Leaf area (cm <sup>2</sup> )	SLA (cm <sup>2</sup> g <sup>-1</sup> )	RWC (%)	Succulence (g FM cm <sup>-2</sup> )
0	40	66.53 $\pm$ 16.19 c <sup>1</sup>	133.67 $\pm$ 19.13 b	72.47 $\pm$ 16.95 c	88.18 $\pm$ 7.48 ab	0.51 $\pm$ 0.04 b
0	55	99.87 $\pm$ 7.16 b	139.12 $\pm$ 20.00 b	96.47 $\pm$ 12.95 c	88.59 $\pm$ 5.39 ab	0.73 $\pm$ 0.15 a
100	40	62.58 $\pm$ 15.65 c	110.83 $\pm$ 23.51 c	125.88 $\pm$ 19.58 b	82.41 $\pm$ 7.33 b	0.56 $\pm$ 0.05 b
100	55	138.09 $\pm$ 16.23 a	243.34 $\pm$ 16.47 a	178.97 $\pm$ 53.94 a	94.22 $\pm$ 8.59 a	0.57 $\pm$ 0.06 b
Salinity		**2	***	***	ns	ns
Age		***	***	**	*	***
Salinity * Age		***	***	ns	ns	**

<sup>1</sup>Means ( $n=6$ )  $\pm$  SD were compared with Duncan test. Within each column, values followed by a common letter are not significantly different ( $p \leq 0.05$ ).

<sup>2</sup>Means  $\pm$ SD ( $n=6$ ) significance at  $P < 0.05$  according to ANOVA test, not significant at  $P > 0.05$  (ns), significant at  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*). DAT: Days after transplanting

**Tab. 2:** Foliar concentrations of macronutrients in ice plant grown under non-saline (0 mM NaCl) and saline (100 mM NaCl) conditions, 40 or 55 days after transplanting.

NaCl (mM)	Age (DAT)	C (mg g <sup>-1</sup> DM)	N (mg g <sup>-1</sup> DM)	P (mg g <sup>-1</sup> DM)	K (mg g <sup>-1</sup> DM)	Ca (mg g <sup>-1</sup> DM)	Mg (mg g <sup>-1</sup> DM)
0	40	373.91 ± 35.75 ab <sup>1</sup>	64.18 ± 3.79 a	4.20 ± 0.46 a	75.05 ± 13.50 a	4.84 ± 0.85 b	13.26 ± 1.32 b
0	55	431.17 ± 119.95 a	56.39 ± 15.78 ab	2.70 ± 0.83 b	43.78 ± 6.29 b	7.55 ± 1.11 a	18.49 ± 3.49 a
100	40	322.22 ± 53.30 b	52.66 ± 9.68 b	4.29 ± 0.90 a	81.28 ± 9.49 a	2.53 ± 0.47 c	10.90 ± 1.20 b
100	55	370.33 ± 57.40 ab	45.19 ± 7.31 b	2.55 ± 0.66 b	31.82 ± 4.66 c	7.98 ± 1.78 a	17.98 ± 3.60 a
Salinity		ns <sup>2</sup>	**	ns	ns	*	ns
Age		ns	ns	***	***	***	***
Salinity * Age		ns	ns	ns	*	**	ns

<sup>1</sup>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).

<sup>2</sup>Means ±SD (n= 6) significance at P<0.05 according to ANOVA test, not significant at P>0.05 (ns), significant at P<0.05 (\*), P<0.01 (\*\*), and P<0.001 (\*\*\*). DAT: Days after transplanting DM: Dry mass

**Tab. 3:** Foliar concentrations of micronutrients in ice plant grown under non-saline (0 mM NaCl) and saline (100 mM NaCl) conditions, 40 or 55 days after transplanting.

NaCl (mM)	Age (DAT)	Mn (µg g <sup>-1</sup> DM)	Zn (µg g <sup>-1</sup> DM)	Fe (µg g <sup>-1</sup> DM)	Na (mg g <sup>-1</sup> DM)
0	40	107.91 ± 29.18 d <sup>1</sup>	64.72 ± 16.44 a	1509.37 ± 339.51 b	4.04 ± 0.46 c
0	55	483.07 ± 66.04 a	67.06 ± 1.79 a	1666.48 ± 474.77 b	3.41 ± 1.02 c
100	40	194.63 ± 10.26 c	49.78 ± 14.45 b	471.32 ± 294.84 c	75.82 ± 6.32 a
100	55	320.47 ± 37.30 b	65.60 ± 5.52 a	2305.05 ± 494.23 a	24.01 ± 7.72 b
Salinity		* <sup>2</sup>	ns	*	***
Age		***	ns	***	***
Salinity * Age		***	ns	***	***

<sup>1</sup>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).

<sup>2</sup>Means ±SD (n= 6) significance at P<0.05 according to ANOVA test, not significant at P>0.05 (ns), significant at P<0.05 (\*), P<0.01 (\*\*), and P<0.001 (\*\*\*). DAT: Days after transplanting DM: Dry mass

and the plants that presented a higher Na concentration were the 40 DAT plants treated with salt (Age, p<0.001; Salinity\*Age, p<0.001).

## Discussion

In response to the hypothesis raised in this work, we assume that the effect attributed to salinity depended in a significant way on the age of the plant according to our results. Salinity affected differently shoot DM and shoot height of *Mesembryanthemum*, which had no effect in the 55 DAT plants, while in 40 DAT ones, 100 mM saline treatment reduced these growth parameters with respect to its controls. This could be due to the fact that 55 DAT plants are better adapted to salinity than 40 DAT plants according to HERPPICH et al., (1992). However, salinity did not produce differences in root DM or root length despite the fact that, as we expected, both root DM and length were lower in 40 DAT plants. Thus, root growth parameters indicated that salinity would not have indirect effects on decreased water and nutrient absorption in *M. crystallinum*.

In terms of fresh leaf biomass and leaf area, a moderate salinity was beneficial for the 55 DAT ice plant, since the maximum FM and area values of the leaves were obtained with the irrigations with 100 mM NaCl according to previous work (RODRÍGUEZ-HERNÁNDEZ and GARMENDIA, 2022b). However, in the 40 DAT plants, this effect was not significant in the case of the leaf FM and salinity decreased leaf area. Our results in leaf fresh production are in accordance with

HERPPICH et al. (2008) who reported that a moderate saline treatment affected *M. crystallinum* growth only in plants older than 105 days and did not affect younger plants. This different behaviour depending on the age of the plants could be partially explained by the increase in RWC in 55 DAT plants irrigated with NaCl, although it was not significant and did not occur in the case of the 40 DAT plants. Similarly, HERPPICH et al. (2008) described that a moderate saline treatment did not significantly affect mean leaf water content between plants of different age. The results can be explained by the adaptative mechanisms to the saline stress that *Mesembryanthemum crystallinum* has, since the bladder cells accumulate water, in addition to salts, to improve ionic and osmotic stress (AGARIE et al., 2007) and the CAM metabolism causes the plant to lose less water, due to stomatal closure during the day (HERPPICH et al., 2008). In the same line were the results of ATZORI et al. (2017), in which more mature ice plants increased their biomass and area when they were irrigated with moderate salinity, suggesting an extension of vegetative period due to salinity. Also, it was observed a significant increase in SLA with salinity, this may be due to the increase in leaf area under saline conditions in 55 DAT plants, although of the fact that 40 DAT plants with greater area were the controls. On the contrary, ATZORI et al. (2017) found no significant differences in this parameter. In any case, salinity produced an improvement in growth in 55 DAT plants, in important variables for the productivity of glacier lettuce, such as the fresh leaf biomass or area, when the salt concentration used was 100 mM NaCl. HERPPICH et al. (2008) indicated that the

repetitive irrigation with salinity did not negatively affect the growth of the plant, as in WINTER (1973), in which the maximum growth was obtained with a concentration of 100 mM NaCl. In fact, ATZORI et al. (2017) observed growth reduction only in the youngest stage of plants and with high salinity level. Similarly, HERPPICH et al. (1992) obtained that in 10 DAT plants, still in development, salinity caused a delay in their phenology. It was suggested that the adaptive mechanisms that make glacier lettuce tolerant against salinity, bladder cells and C3-CAM facultative metabolism, are not fully developed in younger plants, so they are more sensitive to salinity. Our results corroborated that at the beginning of the cultivation of the ice plant, the concentration of salts in the irrigation water should be adjusted, being able to increase salinity in stages over two months after plant transplanting.

Due to the adaptations to salinity developed in *M. crystallinum*, it was expected that the plant had greater succulence under salinity treatment. On the contrary, in this work and in discordance with ATZORI et al. (2017), 55 DAT ice plants had greater succulence in the control. Since succulence is calculated as the ratio of leaf FM between the leaf area, the increase in leaf area caused by saline conditions in 55 DAT plants would explain a decrease in succulence. However, in 40 DAT plants there were no significant differences in water status between the control plants and those treated with NaCl. ADAMS et al. (1998) found that juvenile ice plants have a smaller number and size of bladder cells, which would explain that the results are different depending on the age of the plant.

In relation to photosynthetic pigments, salinity had no detrimental effects on the concentration of chlorophylls, and even in both 40 and 55 DAT plants, was higher in the plants treated with salinity. According to ARGENTEL et al. (2006), the halophilic plant *M. crystallinum* increased the activity of the chlorophyllase enzyme, which affects the synthesis of chlorophylls under saline conditions. HERPPICH et al. (2008) explained the increase in chlorophyll by a general physiological stimulation in response to salinity. Furthermore, BARKER et al. (2004) observed that salinity did not decrease the concentration of carotenoids as in our work, since in both 40 and 55 DAT plants the concentration of carotenoids was slightly higher in plants irrigated with 100 mM NaCl than in the controls. These results coincide with those obtained by ATZORI et al. (2017) where the concentration of carotenoids increased with the increase in salinity.

Salinity did not influence the concentration of C, P and Mg independently of the date when the saline condition was imposed. Otherwise, as expected, the addition of NaCl increased the foliar concentration of Na in ice plants, demonstrating that *M. crystallinum* acts as a sequestrant of salts, due to the presence of bladder cells (AGARIE et al., 2007). Moreover, ADAMS et al. (1998) found that in mature leaves more Na is concentrated than in young and that young leaves have a smaller number and size of bladder cells. This research suggests less compartmentalization of salts in bladder cells of young leaves, which would cause toxic effects to the plant and explain the worst results in growth parameters as leaf FM with salinity in 40 DAT plants. Our results showed that leaves of 55 DAT plants accumulated less Na, probably related to a dilution effect associated to the increase of their leaf biomass production.

Potassium, Ca and Mg are antagonists of Na, thus in plants not adapted to salinity a decrease in their concentration would be expected, due to a displacement by Na from the cell membrane binding sites (NIU et al., 1995; DODD et al., 2010). However, Ca and Mg remained invariable with salinity in 55 DAT plants and K in 40 DAT ones, while the concentration of K decreased with salinity in 55 DAT plants and Ca in 40 DAT plants. In the study by ATZORI et al. (2017) Na was accumulated in ice plants irrigated with salt, while K decreased with increasing salinity. These results are in the same line as those obtained in our study in 55 DAT plants. In addition, 40 DAT plants subjected to 100 mM NaCl, the foliar Mn concentration increased and

N, Ca, Zn and Fe decreased. Perhaps the decrease of some of these minerals under saline conditions, was another factor that caused the depletion of some growth parameters in 40 DAT plants.

## Conclusions

The favourable results in the growth parameters such as greater fresh foliar mass and area in 55 DAT plants, indicate that ice plant can not only be a suitable crop for breeding under moderately saline conditions (100 mM), but also stimulate the development of its edible part. Furthermore, parameters as SLA, RWC, pigments and nutrients as C, N, P, Ca, Mg, Zn and Fe remained unchanged or enhanced in 55 DAT plants treated with salinity, showing that these plants maintain or increase its nutritional quality under these conditions. However, in 40 DAT plants the adaptive mechanisms seem to be not fully developed, so they show more sensitive to salinity. According to our results, the implementation of the cultivation of glacier lettuce under greenhouse conditions could be performed with moderately salinized water resources, especially in plants over 8 weeks of growth.

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## Conflicts of interest

No potential conflict of interest was reported by the authors.

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