Research Article

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Optimum growth and quality of the edible ice plant under saline conditions

María del Carmen Rodríguez-Hernández* and **Idoia Garmendia**

Abstract

BACKGROUND: Ice plant is a halophyte, known for its antioxidant activity and for being a highly functional food. It is capable of increasing its contents of health-promoting compounds when subjected to certain stresses such as salinity. The objective of this work was to determine the plant's best growing conditions to achieve both an optimal production of bioactive metabolites and high crop yield. *Mesembryanthemum crystallinum* were grown under semi-controlled conditions and four saline treatments were applied at: 0, 100, 200 and 300 mmol L^{-1} sodium chloride (NaCl), respectively.

RESULTS: The 100 mmol L^{-1} NaCl treatment induced a slight increase in shoot dry weight (DW) and enhanced the leaf area. At higher salinity levels, however, the shoot biomass decreased. The concentration of starch and total proteins declined as the concentration of salt increased, while the total soluble sugars (TSS) content was lower in 100 and 300 mmol L^{-1} NaCl treatments. Proline increased in conditions over 100 mmol L^{-1} NaCl. Furthermore, plants grown with 300 mmol L^{-1} of NaCl presented the highest values of glutathione, ascorbic acid and vitamin C. Antioxidant enzymes activity and total phenolics increased with the severity of the salinity.

CONCLUSION: Ice plant accumulates high levels of health-promoting compounds when grown with 300 mmol L^{-1} NaCl. A high concentration of beneficial compounds, however, is detrimental to the plant's growth. Moreover, 100 mmol L^{-1} NaCl treatment not only improved the concentration of bioactive and antioxidant compounds but also preserved the crop yield. It could thus be interesting to promote the cultivation of this high nutritional value plant in environments of moderate salinity.

Keywords: *Mesembryanthemum crystallinum*; salt stress; antioxidant properties; food; yield

INTRODUCTION

The common ice plant, also known as glacier lettuce (*Mesembryanthemum crystallinum* L.), is an annual plant belonging to the Aizoaceae family. A native of the Namibi desert in southern Africa, it is widely distributed and naturalized in Western Australia, south-western United States, Mexico's and Chile's Pacific coast,[1] and Europe's coastal areas.[2] It is already being consumed as a vegetable crop in several regions including India, California, Australia and New Zealand and in some European countries,[3] that is, Germany[4] and The Netherlands.[5]

Mesembryanthemum crystallinum is well known for its antioxidant activity which can detoxify reactive oxygen species (ROS). It is also recognized for its ability to rapidly accumulate phytochemicals and secondary metabolites, including: carotenoids (beta-carotene), pinitol, betacyanin, phenolic compounds, and flavonol conjugates.[3,6-10] Carotenoids are regarded as effective antioxidants[11] while phenolic compounds have anticancer and antioxidant properties,[12,13] exert chelating effects, modulate the activity of various enzyme systems[14,15] and have health benefit.[16,17] Moreover, *M. crystallinum* is characterized by the presence of antioxidant enzymes such as ascorbate peroxidase, superoxide dismutase and catalase.[18] It is also classified as a highly functional food given its high concentration of polyols.[3] For the reasons given, *M. crystallinum* is a traditional medicine. It is recognized in particular for: its demulcent and diuretic effects[19]; its role in protecting skin against radiation given its significant content of antioxidant molecules[20]; and its antiseptic properties.[21] Moreover, *M. crystallinum* is used to treat ocular infections and has become a good candidate for pharmaceutical and cosmetic applications.[3,7,22]

Added to its high nutraceutical value, *M. crystallinum* is an annual facultative halophyte with a crassulacean acid metabolism (CAM), which among others, is induced by saline stress. It is thus highly tolerant to salinity and can complete its life cycle in soil with an equivalent concentration of sodium chloride (NaCl) than seawater.[2] This characteristic is particularly relevant today. Indeed, soil salinity is a major, global, issue[23,24] owing to its adverse impact on agricultural productivity and sustainability.[25] Furthermore, soil salinization is projected to increase in future climate change scenarios led by rising sea levels, the impact on coastal areas, and temperature increases. The latter will inevitably

* *Correspondence to: M del Carmen Rodríguez-Hernández, Department of Earth and Environmental Sciences, University of Alicante, PO Box 99, Alicante 03080, Spain. E-mail: maricarmen.rodriguez@ua.es*

Department of Earth and Environmental Sciences, University of Alicante, Alicante, Spain

result in higher evaporation levels and further salinization.[25] This global problem can be solved by developing tolerant crops, reclaiming degraded saline soil and/or domesticating wild plants with desirable properties.[26] In this context, several facultative halophytic members of the Aizoaceae have been used as an alternative crop for many years.[4] In addition, it is well known that abiotic stresses such as salinity can enhance the bioactive compounds in certain plant species that can benefit and improve human health.[23,27] Examples include the concentration of polyols, which increases under saline and water stress conditions,[3] or different bioactive compounds which were affected by light quality and intensity.[28]

In this light, this highly nutritional plant is worthy of study and notably the growing conditions that may bring about an increase in its health-promoting properties. Therefore, the objective of this work was to analyse the antioxidant response of the glacier lettuce grown under increasingly saline conditions. We also sought to determine the best balance between optimal crop production and high bioactive compound contents.

MATERIALS AND METHODS

Plant material and growth conditions

Mesembryanthemum crystallinum L. seeds were collected from wild plants in Alicante (southeast Spain) and, disinfected using 0.5% of sodium hypochlorite (NaClO) for 2 h. They were then pre-hydrated with aerated, deionized water for 22 h. Subsequently, they were germinated in vermiculite hydrated with deionized water and maintained in a growth chamber at an air temperature (*T*) of 24 °C day/night (D/N) and relative humidity (RH) of 70% D/N.[29] The chamber's light conditions 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 36 W/83 Germany and Silvana F36W/GRO, Wilmington, Massachusetts, USA). After 20 days, the seedlings were transferred to a glasshouse under semi-controlled conditions of *T* D/N: 25/18 °C; RH D/N: 60/80% and received natural daylight (at a mean photosynthetic photon flux rate of 400 μmol m^{-2} s^{-1}).[30] They were then transplanted into 1 L plastic pots containing vermiculite and watered weekly with 500 mL of Hoagland solution. A total of 55 days after their transplantation, the plants were divided into four homogeneous groups of six seedlings. Four saline treatments were applied: control (0 mmol L^{-1}), 100, 200 and 300 mmol L^{-1} 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 under saline conditions, the plants were harvested and the different determinations were performed. At the end of the experiment, the electrical conductivity (EC) of the substrate from the non-saline container and the pots cultivated under 100, 200 and 300 mmol L^{-1} of NaCl was 1.80, 13.67, 19.08 and 25.01 dS m^{-1}, respectively.

Growth parameters

The production of the plant's edible part was measured based on leaf fresh weight (FW) and shoot dry weight (DW) determined after drying fresh matter at 80 °C in an oven until reaching a constant weight. Leaf area was estimated using the app 'Easy Leaf Area Free'.[31]

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.

Starch, total soluble sugars (TSS), proline and proteins in leaves were quantified in potassium phosphate buffer (KPB) (50 mmol L^{-1}, pH 7.5) extracts of fresh tissue (0.1 g). These extracts were filtered through four cheesecloth layers and centrifuged at 28 710 × *g* for 15 min at 4 °C. The pellet was used to determine the starch.[32] The supernatant was collected and stored at 4 °C to determine TSS, proline and proteins. TSS were spectrophotometrically analysed with the anthrone reagent.[33] Free proline was estimated via spectrophotometric analysis at 515 nm of the ninhydrine reaction.[34] Total soluble proteins were measured following the Bradford method[35] using bovine serum albumin (BSA) as a standard.

Total glutathione (measured as total thiol) and vitamin C were extracted according to Ruiz and Blumwald.[36] The leaf tissue was inserted in 6% *m*-phosphoric acid (pH 2.8) containing 1 mmol L^{-1} EDTA. The extracts were centrifuged at 15 000 × *g* for 10 min at 4 °C. The supernatant was collected to analyse the glutathione and vitamin C. Total glutathione was measured in a reaction mixture consisting of: 400 μL of reagent A [110 mmol L^{-1} $Na_2HPO_4{\cdot}7H_2O$, 40 mmol L^{-1} $NaH_2PO_4{\cdot}H_2O$, 15 mmol L^{-1} EDTA, 0.3 mmol L^{-1} 5,5-dithiobis(2-nitrobenzoic acid)], 320 μL reagent B [1 mmol L^{-1} EDTA, 50 mmol L^{-1} imidazole, an equivalent of 1.5 units of glutathione reductase activity (baker's yeast, Type III)], and 400 μL of a 1:50 dilution of the leaf extract in 5% disodium hydrogen phosphate (Na_2HPO_4) (pH 7.5) prepared immediately prior to starting the assay. The reaction was initiated by adding 80 μL of NADPH and the absorbance changes measured at 412 nm.

The contents of ascorbic acid, dehydroascorbic and vitamin C were determined using the high-performance liquid chromatography (HPLC) technique (uHPLC 1260 Infinity Binary LC System, Agilent Technologies, Santa Clara, CA, USA).[37] Vitamin C comes in two biologically active forms: its reduced form, or ascorbic acid; and its oxidized form, or dehydroascorbic acid. A 20 μL volume of sample or standard was injected into a stainless steel, C18 (5 μm) column (Lichrospher, Agilent Technologies) operated at ambient temperature. A 0.2 mol L^{-1} potassium dihydrogen phosphate (KH_2PO_4) in deionized water solution was used as the mobile phase with a flow rate of 0.5 mL min^{-1}. The pH of the mobile phase was adjusted to 2.4 by phosphoric acid (H_3PO_4). An ultraviolet-visible (UV-vis) detector at 254 nm at 30 °C was used. The calibration curve was constructed with ascorbic acid at concentrations between 10 to 100 mg L^{-1}, with 1 mg mL^{-1} of DTT. To quantify the ascorbic acid, the previously diluted extract was injected into the HPLC. To quantify the vitamin C (ascorbic acid + dehydroascorbic acid), 1 mL of the previously diluted extract was taken and 1 mg of DTT was added. The mixture was allowed to react for 2 h in the dark and was injected into the HPLC. Dehydroascorbic acid was calculated based on the difference between total vitamin C content and ascorbic acid.

The concentration of leaf carotenoids was determined following Sesták *et al.*[38] The samples (20 mg FW) were included in 5 mL of 96% ethanol at 80 °C for 10 min to extract the carotenoids. The absorbance of the extracts was spectrophotometrically measured and the equations reported by Lichtenthaler[39] were used to calculate the concentration of carotenoids.

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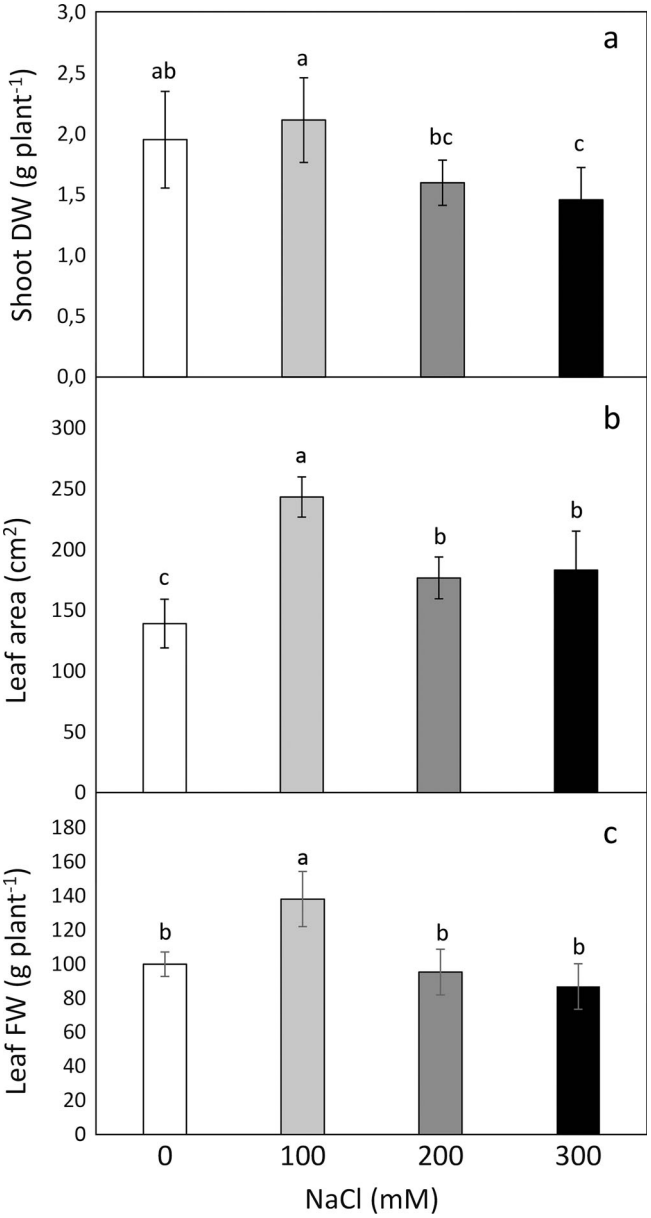

Figure 1. Shoot DW (a), leaf area (b) and leaf FW (c) in ice plant subjected to different salt conditions. Means ($n = 6$) ± SD were compared with Duncan test. Bars followed by a common letter are not significantly different ($P \leq 0.05$). DW: dry weight; FW: fresh weight.

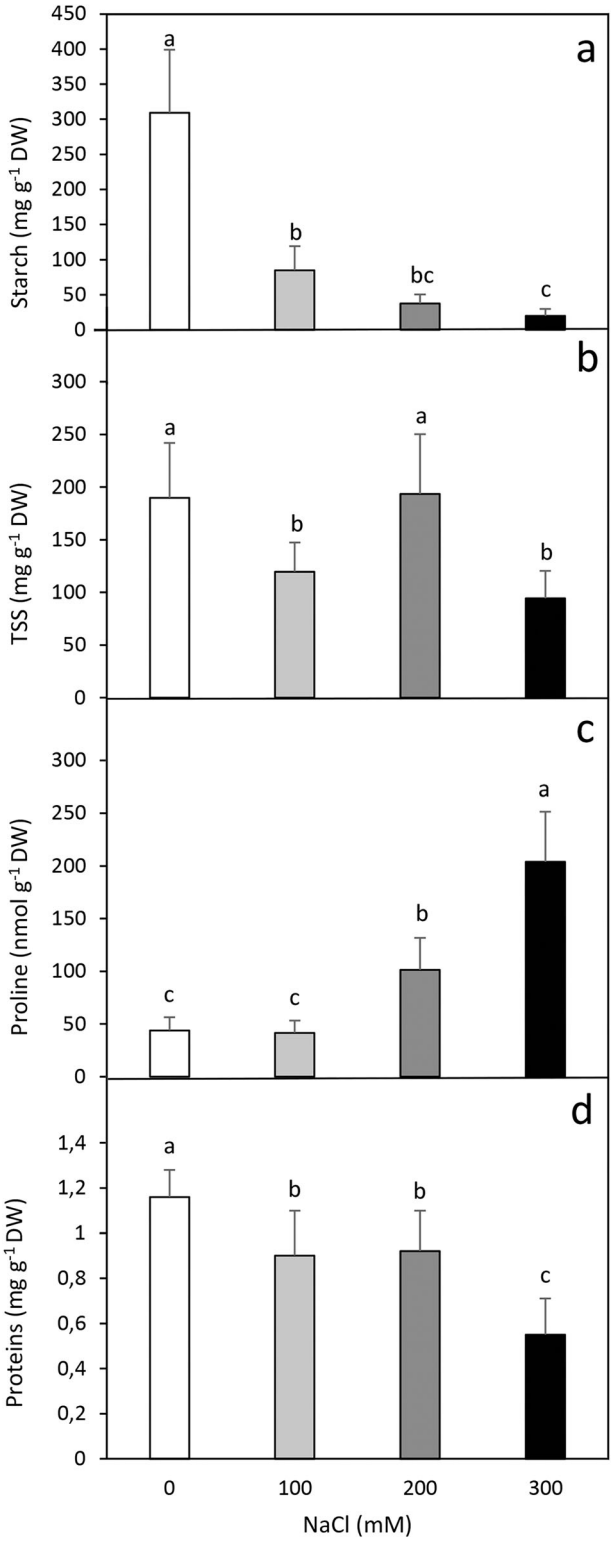

Figure 2. Leaf concentrations of starch (a), total soluble sugars (TSS) (b), proline (c) and proteins (d) in ice plant subjected to different salt conditions. Means ($n = 6$) ± SD were compared with Duncan test. Bars followed by a common letter are not significantly different ($P \leq 0.05$).

To conduct the extraction of antioxidant enzymes and the subsequent catalase, ascorbate peroxidase and glutathione reductase activity assays, frozen leaf samples (0.4 g) were extracted in 8 mL of 0.1 M KPB (pH 7.0), containing 0.1 mmol L^{-1} EDTA and 1% (*w/v*) polyvinylpolypyrrolidone (PVPP). The homogenate was filtered through four cheesecloth layers and centrifuged at 34 452 × *g* for 10 min at 4 °C. The supernatant was divided into aliquots and stored at 80 °C for further analysis. All the earlier operations were carried out at 5 °C. The catalase (EC1.11.1.6) activity was determined according to Aeb[40] with minor modifications. The reaction mixture (2.1 mL) consisted of 50 mmol L^{-1} KPB (pH 7.0), 10 mmol L^{-1} hydrogen peroxide (H_2O_2) and a 100 µL enzyme extract. The reaction was started by adding H_2O_2 and the decrease in A240 produced by the H_2O_2 breakdown was recorded. Ascorbate peroxidase (APX) (EC 1.11.1.11) was determined following the indications of Nakano and Asada,[41] with slight modifications. The reaction medium

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(2.1 mL) consisted of 80 mmol L^{-1} KPB (pH 7.0 and 0.1 mmol L^{-1} EDTA), 0.75 mmol L^{-1} ascorbate, 0.5 mmol L^{-1} H_2O_2 and 100 µL enzyme extract. The reaction was started by adding H_2O_2 and ascorbate oxidation was determined by the decrease in A290. Glutathione reductase (GR) (EC 1.6.4.2) was assayed as described by Schaedle and Bassham[42] with a reaction medium (2 mL) containing 50 mmol L^{-1} Tris–hydrochloric acid (HCl) (pH 7.5), 3 mmol L^{-1} magnesium chloride ($MgCl_2$), 0.5 mmol L^{-1} oxidized glutathione (GSSG), 3 mmol L^{-1} $NADPH_2$ and 200 µL enzyme extract. The oxidation of $NADPH_2$ was recorded at 340 nm. Enzymatic analyses were performed on the youngest fully mature leaves.

Total phenolic compounds were extracted according to the method of Chapuis-Lardy *et al.*[43] with some modifications. Fresh leaf samples (1 g) were pulverized in liquid nitrogen, mixed with 20 mL of 80% methanol, and homogenized at room temperature for 1 min. After filtration, 0.5 mL of each sample was mixed with 10 mL of distilled water. Total phenolic content was determined from aqueous solutions using spectrophotometric analysis at 760 nm with the Folin–Ciocalteu reagent.[44] Results were expressed as milligrams of gallic acid per gram of DW.

Sodium concentration was determined from leaf samples (0.5 g DW) dry-ashed and dissolved in HCl according to Duque.[45] The analysis was carried out using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, Waltham, MA, 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.

Statistics

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

RESULTS

The results showed that salinity has different effects depending on its concentration. Regarding shoot DW (Fig. 1(a)), 100 mmol L^{-1} NaCl treatment did not significantly influenced this trait, even though a slight increase was observed. In contrast, higher salinity concentrations were significantly detrimental to the shoot's biomass. Leaf area markedly increased under saline conditions (Fig. 1 (b)), this increase being greater when treated with 100 mmol L^{-1} NaCl. And leaf FW (Fig. 1(c)) rose when treated with 100 mmol L^{-1} of NaCl, while no differences were found between the control and highest salt treatments.

Regarding the biochemical analysis, results showed that while the amount of starch in ice plant leaves diminished due to salt stress (Fig. 2(a)), TSS concentration only declined with 100 and 300 mmol L^{-1} of NaCl treatments (Fig. 2(b)). Proline levels increased in plants subjected to saline conditions over 100 mmol L^{-1} (Fig. 2(c)) and the concentration of proteins decreased as the severity of the salinity improved (Fig. 2(d)).

As shown in Table 1, total glutathione concentration presented significant differences according to the salt treatment (Table 1). Plants grown with 300 mmol L^{-1} of NaCl were found to have the highest glutathione content compared to the rest of saline treatments. In addition, the control treatment presented the lowest glutathione values and there was no difference compared to plants subjected to 100 mmol L^{-1} NaCl.

With respect to the ascorbic acid, dehydroascorbic acid and vitamin C in leaves, Table 1 illustrates how both ascorbic acid

Table 1. Total glutathione, ascorbic acid, dehydroascorbic acid, vitamin C and carotenoids in ice plant subjected to different salt conditions

Treatment [NaCl] (mmol L^{-1})	Glutathione (mmol mg^{-1} DW)	Ascorbic acid (mg kg^{-1} DW)	Dehydroascorbic acid (mg kg^{-1} DW)	Vitamin C (mg kg^{-1} DW)	Carotenoids (mg g^{-1} DW)
0	106.07 ± 43.44 c	0.20 ± 0.10 d	1.40 ± 0.50 a	1.60 ± 0.60 c	5.35 ± 1.10 ab
100	163.16 ± 36.57 bc	1.00 ± 0.30 c	1.20 ± 0.30 ab	2.20 ± 0.60 bc	6.47 ± 1.76 a
200	207.49 ± 34.13 b	2.20 ± 0.30 b	0.40 ± 0.00 b	2.60 ± 0.40 ab	4.22 ± 0.64 bc
300	320.49 ± 68.68 a	2.80 ± 0.10 a	0.50 ± 0.10 b	3.30 ± 0.80 a	3.17 ± 0.88 c

Means ($n = 6$) ± standard deviation were compared with Duncan test. Within each column, values followed by a common letter are not significantly different ($P \leq 0.05$).
NaCl, sodium chloride; DW, dry weight.

Table 2. Catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) in ice plant subjected to different salt conditions

Treatment [NaCl] (mmol L^{-1})	CAT (mmol H_2O_2 mg $protein^{-1}$)	APX (mmol ascorbate mg $protein^{-1}$)	GR (mmol NADPH mg $protein^{-1}$)
0	133.83 ± 26.23 c	1163.36 ± 156.90 c	196.14 ± 46.46 c
100	189.68 ± 36.21 bc	1873.47 ± 381.79 b	361.87 ± 70.85 b
200	244.85 ± 58.77 b	2540.28 ± 771.26 b	464.23 ± 31.96 b
300	453.05 ± 126.16 a	4342.05 ± 752.13 a	964.22 ± 189.89 a

Means ($n = 6$) ± standard deviation were compared with Duncan test. Within each column, values followed by a common letter are not significantly different ($P \leq 0.05$).

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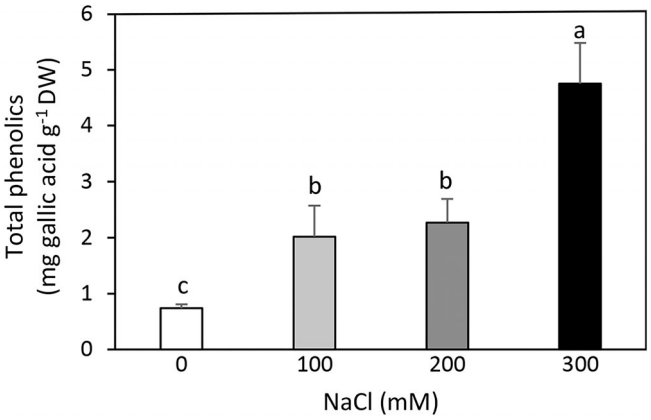

Figure 3. Total phenolics in ice plant subjected to different salt conditions. Means ($n = 6$) ± SD were compared with Duncan test. Bars followed by a common letter are not significantly different ($P \leq 0.05$).

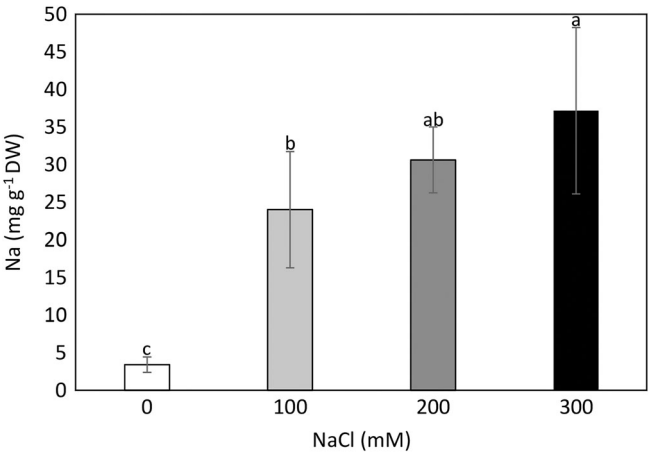

Figure 4. Leaf concentration of Na in ice plant subjected to different salt conditions. Means ($n = 6$) ± SD were compared with Duncan test. Bars followed by a common letter are not significantly different ($P \leq 0.05$).

and vitamin C increased correlatively with the salt concentration, obtaining the highest values the plants subjected to 300 mmol L^{-1} NaCl. An opposite trend was found for the content of dehydroascorbic acid, exhibiting lower values as salinity increased.

In relation to the carotenoids, plants cultivated with 100 mmol L^{-1} NaCl saw their levels slightly rise while 200 and 300 mmol L^{-1} NaCl treatments induced a decrease in carotenoids (Table 1).

The effect of the salinity on antioxidant enzyme activity in ice plant leaves was also assessed (Table 2). Catalase, ascorbate peroxidase and GR activities were greater when the salinity treatment increased, the maximum enhancement occurring with the 300 mmol L^{-1} NaCl treatment.

Similarly, 300 mmol L^{-1} NaCl treatment produced a significantly higher concentration of total leaf phenolics (Fig. 3), while the control treatment presented the lowest values. No differences were found between the results of the 100 and 200 mmol L^{-1} NaCl treatments.

The salinity effect on the ice plant's leaf sodium (Na) concentration was also assessed (Fig. 4). As expected, the accumulation of Na was greater as the severity of salt stress increased.

DISCUSSION

Healthy soil is essential for global food production and maintaining climate sustainability.[46] Inland and coastal cultivation of halophytes will contribute to the establishment of innovative farming systems in marginal lands. The latter, in turn, enables to significantly increase the productivity and quality of commercial crops typical of the area and to reduce soil salinization. It also allows to identify new salt-tolerant cash crops that can be cultivated under the extreme conditions of such lands.[47] In this work, it has been shown that it was possible to enhance the growth of the halophyte *M. crystallinum* by applying an appropriate rate of salinity (100 mmol L^{-1} NaCl). An increase both of the leaf biomass and leaf area was achieved compared to the control treatments. It would thus seem that growth can be somewhat stimulated by applying this concentration of salt in ice plants under pot culture in semi-controlled glasshouse conditions. Moreover, the higher salinity treatments produced a greater leaf area compared to the control treatment, even though shoot DW diminished. This finding is significant, as leaves are the edible part to be harvested for fresh consumption.[5] Recently, ice plants have also been successfully incorporated as dry powder in cookies, replacing wheat flour.[48]

Halophytes, as ice plants, balance their growth rate with their needs in salt for osmotic adjustments.[49] The salt tolerance of halophytes is often associated with several osmotic adjustments that lead to the accumulation of a number of organic solutes. Examples include soluble sugars[50,51] or proline,[5] other free amino acids, proteins and several effectors.[15,24,52] According to our results, ice plant did not seem to use soluble sugars as solutes to cope with salinity. This finding is compatible with that of Atzori *et al.*,[5] who observed a relationship between decreasing soluble sugars and increasing saline conditions. This previous work indicated that other solutes, such as proline and polyols, act as osmoregulatory metabolites. Therefore, the halophyte plant's production of a high carbohydrate concentration in response to salinity may not be necessary.[5] In this sense, our results support this suggestion, as the proline concentration increased when salinity exceeded 100 mmol L^{-1} of NaCl, the greatest values being achieved when treated with high salt levels. Thus, plants treated with a higher saline concentration would perform a greater osmotic adjustment to alleviate salinity's negative effect. This proline accumulation in response to salinity could be at the detriment of starch and proteins, which balance osmotic adjustment and growth. In fact, shoot DW decreased under high salinity treatment. Stress-induced accumulation of proline in the common ice plant has been associated with both, osmoprotectory and antioxidant functions.[53] Likewise, according to previous studies, the carotenoids content, which is involved in energy adjustment and the transport of electron in the photosystem,[54] decreased in plants treated with over 100 mmol L^{-1} of NaCl.[55-57] 57 This supports the suggestion that high salinity is negatively affecting the photosynthetic process, and consequently plant growth.

However, the ice plant is known as a functional food.[58-60] It is rich in dietary antioxidant compounds (polyphenolic compounds, vitamin, and ascorbic acid) and endogenous antioxidant enzymes which protect the organism against oxidation damage.[61] In this work, improvements were found regarding both ascorbic acid and vitamin C in *M. crystallinum* leaves that were cultivated with salinity compared to the control treatments. These results are in agreement with previous results on ice plants[62] and other

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halophytes,[63] but opposite to the effects of salinity on vitamin C in many glycophytes such as coriander, pepper and tomato.[23,27,64] Nevertheless, the glutathione content obtained in ice plant leaves increased with salinity. Glutathione synthesized under conditions of saline stress has been correlated with tolerance to salinity.[35,65,66]

Furthermore, as a part of the antioxidant response system, plants subjected to salinity increased the activity of the antioxidant enzyme catalase. Catalase plays a role in maintaining the cell's redox homeostasis,[67] so it requires greater activity in plants subjected to severe saline stress as in the case described in this work. In addition, salinity enhanced the activities of both ascorbate peroxidase and GR in glacier lettuce leaves. These two enzymes are essential for maintaining the redox-states of ascorbate and glutathione, probably as a part of the antioxidative defence system against salt treatment.[68] These results would be in accordance with the vitamin C and glutathione concentrations quantified in this work, which increased with the level of salinity.

Phenolic compounds, as in the case of ascorbic acid, serve as non-enzymatic antioxidants, that increase a cell's antioxidant capacity to scavenge ROS produced under salinity stress.[69,70] This result is in agreement with the findings of the present work, where leaf phenolics increased under salinity treatments. Previous studies have shown that ice plants have 23 phenolic components, catechin being the most abundant compound, followed by pyrogallol and catechol.[48] In addition, abiotic stress, and salinity in particular, has been shown to induce the accumulation of phenol 2,6-bis (1.1-dimethylethyl)-4-methylphenol (BHT) in *M. crystallinum* leaves, which is primarily used as an antioxidant food additive.[71] Other authors have also found that phenolic compounds play an active role as an antitumour substance.[72] In addition, these components help to improve the ice plant's powder extract, which can be used as food antioxidant and anticancer agents, to protect against certain diseases.[48] Thus, the results of the present study may be particularly significant for the health food industry.

CONCLUSION

This study provides evidence that ice plants cultivated under salt conditions respond differently according to the level of saline concentration. Although salinity improved the ice plant's concentration of bioactive compounds, including its antioxidant properties, high saline conditions were detrimental to the plant's growth. In contrast, a moderate concentration of salinity (100 mmol L^{-1} NaCl) not only improved the accumulation of nutraceuticals, but also enhanced the production of edible leaves. Promoting the cultivation of this highly nutritional plant in moderately saline environments may thus present a specific interest.

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CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1 Bohnert HJ and Cushman J, The ice plant cometh: lessons in abiotic stress tolerance. *J Plant Growth Reg* **19**:334–346 (2000).
2 Adams P, Nelson DE, Yamada S, Chamara W, Jensen RG, Bohnert HJ *et al*., Growth and development of *Mesembryanthemum crystallinum* (Aizoaceae). *New Phytol* **138**:171–190 (1998).
3 Agarie S, Kawaguchi A, Kodera A and Sunagawa H, Potential of the common ice plant, *Mesembryanthemum crystallinum* as a new high-functional food as evaluated by polyol accumulation. *Plan Prod Sci* **12**:37–46 (2009).
4 Herppich WB, Huyskens-Keil S and Schreiner M, Effects of saline irrigation on growth, physiology and quality of *Mesembryanthemum crystallinum* L., a rare vegetable crop. *J Appl Bot Food Qual* **82**:47–54 (2008).
5 Atzori G, Vos AC, Rijsselberghe M, Vignolini P, Rozema J, Mancusoa S *et al*., Effects of increased seawater salinity irrigation on growth and quality of the edible halophyte *Mesembryanthemum crystallinum* L. under field conditions. *Agric Water Manag* **187**:37–46 (2017).
6 Vogt T, Ibdah M, Schmidt J, Wray V, Nimtz M and Strack D, Light-induced betacyanin and flavonol accumulation in bladder cells of *Mesembryanthemum crystallinum*. *Phytochemistry* **52**:583–592 (1999).
7 Ibdah M, Krins A, Seidlitz HK, München HZ and Heller W, Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. *Plant Cell Environ* **25**:1145–1154 (2002).
8 Ślesak I, Ślesak H, Libik-Konieczny M and Miszalski Z, Antioxidant response system in the short-term post-wounding effect in *Mesembryanthemum crystallinum* leaves. *J Plant Physiol* **165**:127–137 (2008).
9 Hanen F, Riadh K, Samia O, Sylvain G, Christian M and Chedly A, Interspecific variability of antioxidant activities and phenolic composition in *Mesembryanthemum genus*. *Food Chem Toxicol* **47**:2308–2313 (2009).
10 Weeplian T, Yen TB and Ho YS, Growth, development, and chemical constituents of edible ice plant (*Mesembryanthemum crystallinum* L.) produced under combinations of light-emitting diode lights. *HortScience* **53**:865–874 (2018).
11 Young AJ and Lowe GM, Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys* **385**:20–27 (2001).
12 Yao LH, Jiang YM, Shi J, Tomás-Barberán FA, Datta N, Singanusong R *et al*., Flavonoids in food and their health benefits. *Plant Foods Hum Nutr* **59**:113–122 (2004).
13 Alia M, Ramos S, Mateos R, Granado-Serrano AB, Bravo L and Goya L, Quercetin protects human hepatoma HepG2 against oxidative stress induced by tert-butyl hydroperoxide. *Toxicol Appl Pharmacol* **212**:110–118 (2006).
14 Tohidloo G, Souri MK and Eskandarpour S, Growth and fruit biochemical characteristics of three strawberry genotypes under different potassium concentrations of nutrient solution. *Open Agric* **3**:356–362 (2018).
15 Souri MK and Hatamian M, Aminochelates in plant nutrition; a review. *J Plant Nutr* **42**:67–78 (2019).
16 Gee JM and Johnson IT, Polyphenolic compounds: interactions with the gut and implications for human health. *Curr Med Chem* **8**: 1245–1255 (2001).
17 Balasundaram G, Sato M and Webste TJ, Using hydroxyapatite nanoparticles and decreased crystallinity to promote osteoblast adhesion similar to functionalizing with RGD. *Biomaterials* **27**:2798–2805 (2006).
18 Ślesak I, Miszalski Z, Karpinska B and Niewiadomska E, Redox control of oxidative stress responses in the C3-CAM intermediate plant *Mesembryanthemum crystallinum*. *Plant Physiol Biochem* **40**:669–677 (2002).
19 Bouftira I, Hizem H, Mahmoud A, Abdelly C and Sfar S, Effect of *Mesembryanthemum crystallinum* extract against DMH-induced colon carcinogenesis in experimental animals. *Int J Pharma Bio Sci* **3**:1038–1043 (2012).
20 Bouftira I, Abdelly C and Sfar S, Characterization of cosmetic cream with *Mesembryanthemum crystallinum* plant extract: influence of formulation composition on physical stability and antioxidant activity. *Int J Cosmet Sci* **30**:443–452 (2008).
21 Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A *et al*., Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. *C R Biol* **331**:865–873 (2008).
22 Falleh H, Riadh K, Medini F and Guyot S, Antioxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. *Ind Crops Prod* **34**:1066–1071 (2011).
23 Ahmadi M and Souri MK, Growth and mineral elements of coriander (*Corianderum sativum* L.) plants under mild salinity with different salts. *Acta Physiol Plant* **40**:94–99 (2018).

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24 Souri MK and Tohidloo G, Effectiveness of different methods of salicylic acid application on growth characteristics of tomato seedlings under salinity. *Chem Biol Technol Agric* **6**:26 (2019).
25 Shahid SA, Zaman M and Heng L, *Soil Salinity: Historical Perspectives and a World Overview of the Problem, in Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques*. Springer, Cham (2018).
26 Nikalje GC, Bhaskar SD, Yadav K and Penna S, Halophytes: prospective plants for future, in *Ecophysiology, Abiotic Stress Responses and Utilization of Halophytes*, ed. by Hasanuzzaman M, Nahar K and Öztürk M. Springer, Singapore 221–234 (2019).
27 Ahmadi M and Souri MK, Nutrient uptake, proline content and antioxidant enzymes activity of pepper (*Capsicum annuum* L.) under higher electrical conductivity of nutrient solution created by nitrate or chloride salts of potassium and calcium. *Acta Sci Polon Hort Cultus* **18**:113–122 (2019).
28 Kim YJ, Kim HM, Kim HM, Jeong BR, Lee HJ, Kim HJ *et al*., Ice plant growth and phytochemical concentrations are affected by light quality and intensity of monochromatic light-emitting diodes. *Hortic Environ Biotechnol* **59**:529–536 (2018).
29 Thomas J, Malick F, Endreszl C, Davies E and Murray K, Distinct responses to copper stress in the halophyte *Mesembryanthemum crystallinum*. *Physiol Plant* **102**:360–368 (1998).
30 Agarie S, Shimoda T, Shimizu Y, Baumann K, Sunagawa H, Kondo A *et al*., Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. *J Exp Bot* **58**:1957–1967 (2007).
31 Easlon HM and Bloom AJ, Easy leaf area: automated digital image analysis for rapid and accurate measurement of leaf area. *Appl Plant Sci* **2**: 1400033 (2014).
32 Jarvis SC and Walker FR, Simultaneous, rapid, pectrophotometric, determination of total starch, amylose and amylopectin. *J Sci Food Agric* **63**:53–57 (1993).
33 Yemm EW and Willis AJ, The estimation of carbohydrates in plant extracts by anthrone. *Biochem J* **57**:508–514 (1954).
34 Irigoyen JJ, Emerich DW and Sánchez-Díaz M, Water stress induced changes in concentration of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiol Plant* **84**:55–60 (1992).
35 Bradford MM, A rapid and sensitive meted for the quantification of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal Biochem* **12**:961–973 (1976).
36 Ruiz JM and Blumwald E, Salinity-induced glutathione synthesis in *Brassica napus*. *Planta* **214**:965–969 (2002).
37 Gokmen V, Kahraman N, Demir N and Acar J, Enzymatically validated liquid chromatographic method for the determination of ascorbic and dehydroascorbic acids in fruit and vegetables. *J Chromatogr A* **881**:309–316 (2000).
38 Sesták Z, Càtsky J and Jarvis P, *Plant Photosynthetic Production: Manual of Methods*, 1st edn. Dr. W. Junk Publishers, Dordrecht (1971).
39 Lichtenthaler HK, Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol* **148**:350–382 (1987).
40 Aebi H, Catalase in vitro. *Methods Enzymol* **105**:121–126 (1984).
41 Nakano Y and Asada K, Hydrogen peroxide is scavenged by ascrbate-specific peroxidase in spinach chloroplast. *Plant Cell Physiol* **22**: 867–880 (1981).
42 Schaedle M and Bassham JA, Chloroplast glutathione reductase. *Plant Physiol* **59**:1011–1012 (1977).
43 Chapuis-Lardy L, Contour-Ansel D and Bernhard-Reversat F, High performance liquid chromatography of water-soluble phenolics in leaf litter of three *eucalyptus hybrids* (Congo). *Plant Sci* **163**:217–222 (2002).
44 Waterman PG and Mole S eds, *Analysis of Phenolic Plant Metabolites. Methods in Ecology*. Blackwell Scientific Publications, Oxford (1994).
45 Duque F, Joint determination of phosphorus, potassium, calcium, iron, manganese, copper and zinc in plants. *An Edafol Agrobiol* **30**:207–229 (1971).
46 Koch A, McBratney AB, Adams M, Field DJ, Hill R, Lal R *et al*., Soil security: solving the global soil crisis. *Glob Policy* **4**:434–441 (2013).
47 Hamed KB and Custódio L, How could halophytes provide a sustainable alternative to achieve food security in marginal lands? in *Ecophysiology, Abiotic Stress Responses and Utilization of Halophytes*, ed. by Hasanuzzaman M, Nahar K and Öztürk M. Springer, Cham, pp. 259–270 (2019).
48 Essa RY and Elsebaie EM, Ice plant as antioxidant/ anticancer and functional food. *World J Dairy Food Sci* **13**:57–62 (2018).
49 Flowers TJ and Yeo AR, Ion relations of plants under drought and salinity. *Aust J Plant Physiol* **13**:75–91 (1986).
50 Parida A, Das AB and Dam P, NaCl stress causes changes in photosyntheticpigments, proteins, and other metabolic components in the leaves of a truemangrove, *Bruguiera parviflora*, in hydroponic cultures. *J Plant Biol* **45**:28–36 (2002).
51 Wang X, Chang L, Wang B, Wang D, Li P, Wang L *et al*., Comparative proteomics of thellungiella halophila leaves fromplants subjected to salinity reveals the importance of chloroplastic starch andsoluble sugars in halophyte salt tolerance. *Mol Cell Proteomics* **12**:2174–2195 (2013).
52 Ahmadi M and Souri MK, Growth characteristics and fruit quality of chili pepper under higher electrical conductivity of nutrient solution induced by various salts. AGRIVITA. *J Agric Sci* **42**:143–152 (2020).
53 Shevyakova NI, Bakulina EA and Kuznetsov VV, Proline antioxidant role in the common ice plant subjected to salinity and paraquat treatment inducing oxidative stress. *Russ J Plant Physiol* **56**:663–669 (2009).
54 Ghassemi-Golezani K and Farhangi-Abriz S, Biochar alleviates fluoride toxicity and oxidative stress in safflower (*Carthamus tinctorius* L.) seedlings. *Chemosphere* **223**:406–415 (2019).
55 Qiu N, Lu Q and Lu C, Photosynthesis, photosystem II efficiency and thexanthophyll cycle in the salt-adapted halophyte *Atriplex centralasiatica*. *New Phytol* **159**:479–486 (2003).
56 Aghaleh M, Niknam V, Ebrahimzadeh H and Razavi K, Salt stress effects ongrowth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. *Biol Plant* **53**:243–248 (2009).
57 Redondo-Gomez S, Mateos-Naranjo E, Figueroa ME and Davy AJ, Salt-stimulation of growth and photosynthesis in an extreme halophyte, Arthrocnemum macrostachyum. *Plant Biol* **12**:79–87 (2010).
58 He J, Qin L, Chong EL, Choong TW and Lee SK, Plant growth and photosynthetic characteristics of *Mesembryanthemum crystallinum* grown aeroponically under different blue-and red-LEDs. *Front Plant Sci* **8**:361 (2017).
59 Loconsole D, Murillo-Amador B, Cristiano G and De Lucia B, Halophyte common ice plants: a future solution to arable land salinization. *Sustainability* **11**:6076 (2019).
60 Nosek M, Kaczmarczyk A, Śliwa M, Jędrzejczyk R, Kornaś A, Supel P *et al*., The response of a model C3/CAM intermediate semi-halophyte *Mesembryanthemum crystallinum* L. to elevated cadmium concentrations. *J Plant Physiol* **240**:153005 (2019).
61 Ibtissem B, Abdelly C and Sfar S, Properties of *Mesembryanthemum crystallinum* and *Carpobrotus edulis* extracts. *Adv Chem Eng Sci* **2**: 359–365 (2012).
62 He J, Koh DJ and Qin L, LED spectral quality and NaCl salinity interact to affect growth, photosynthesis and phytochemical production of *Mesembryanthemum crystallinum*. *Funct Plant Biol* (2021). https://doi.org/10.1071/FP20375.
63 Osman MS, Badawy AA, Osman AI and Latef AA, Ameliorative impact of an extract of the halophyte *Arthrocnemum macrostachyum* on growth and biochemical parameters of soybean under salinity stress. *J Plant Growth Reg* **40**:1245–1256 (2021).
64 Mardanluo S, Souri MK and Ahmadi M, Plant growth and fruit quality of two pepper cultivars under different potassium levels of nutrient solutions. *J Plant Nutr* **41**:1604–1614 (2018).
65 Noctor G, Arisi AC, Jouanin L, Kunert KJ, Rennenberg H and Foyer CH, Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. *J Exp Bot* **49**:623–647 (1998).
66 Kocsy G, Szalai G and Galiba G, Effect of osmotic stress on glutathione and hydroxymethylglutathione accumulation in wheat. *J Plant Physiol* **161**:785–794 (2004).
67 Mullineaux PM, Karpinski S and Baker NB, Spatial dependence for hy-drogen peroxide-directed signalling in light-stressed plants. *Plant Physiol* **141**:1e5 (2006).
68 Parida AK, Das AB and Mohanty P, Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidativeenzymes. *J Plant Physiol* **161**:531–542 (2004).
69 Gill SS and Tuteja N, Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* **48**:909–930 (2010).
70 Kulbat K, The role of phenolic compounds in plant resistance. *Food Sci Biotechnol* **80**:97–108 (2016).
71 Bouftira I, Abdelly C and Sfar S, Identification of a naturally occurring 2,6-bis (1.1-dimethylethyl)-4- methylphenol from purple leaves of the halophyte plant *Mesembryanthemum crystallinum*. *Afr J Biotechnol* **6**:1136–1139 (2007).
72 Gerhäuser C, Klimo K, Heiss E, Neumann I, Gamal-Eldeen A, Knauft J *et al*., Mechanism-based in vitro screening of potential cancer chemopreventive agents. *Mutat Res/Fundam Mol Mech Mutagen* **523**: 163–172 (2003).

