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ORIGINAL ARTICLE

NaCl elicitation enhances metabolite accumulation and stress resilience in *Inula crithmoides* L. shoot cultures: implications for its nutritional and medicinal value

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Abstract

This study explored the impact of sodium chloride (NaCl) elicitation on the accumulation of primary and secondary metabolites and the oxidative stress responses of *Inula crithmoides* L. (golden samphire) in vitro shoot cultures. Elicitation involved applying different concentrations of NaCl (0, 50, 100, and 200 mM) for 4 weeks. This was followed by assessing its impact on plant growth, physiological parameters (pigments, hydrogen peroxide content, total soluble sugars and proteins, and proline), and secondary metabolism (phenylalanine ammonia-lyase activity, shikimic acid, phenolics, flavonoids, and hydroxycinnamic acids) in the shoots. The extracts were also analysed using high-performance liquid chromatography (HPLC). The NaCl elicitation did not affect shoot growth but increased physiological functions such as photosynthesis and oxidative stress management under moderate salinity levels. In addition, NaCl treatments increased the synthesis of soluble sugars and proteins, particularly proline, as well as bioactive phenolics such as gentisic acid, chlorogenic acid, 4-hydroxybenzoic acid, luteolin-7-O-glucoside, and naringenin-7-O-glucoside. The NaCl elicitation in golden samphire shoot cultures offers a significant method for enhancing the production of important nutritional and bioactive compounds. This underscores the species' potential for cultivation in saline environments and provides valuable prospects for its utilization in the health and nutrition sectors.

Key message

NaCl elicitation significantly enhances the accumulation of metabolites, including sugars, proteins, and bioactive phenolic compounds in golden samphire, improving its nutritional and medicinal value.

Keywords Halophytes · Pigments · Proteins · Sugars · Phenolic compounds · Plant tissue culture

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Introduction

Medicinal plants are invaluable sources of bioactive compounds utilized in various industries, such as pharmaceuticals, cosmetics, food additives, and veterinary products (Fierascu et al. 2021). However, relying solely on natural sources presents challenges such as environmental harm, loss of biodiversity, supply inconsistencies due to seasonal variations, and vulnerability to climate change. To tackle these issues, alternative sourcing methods must be explored to balance resource demand with environmental and ethical concerns for long-term sustainability (Caro et al. 2022).

Plant tissue culture provides a sustainable and controlled method for producing bioactive metabolites. Culturing plant cells, tissues, or organs in a controlled environment ensures

year-round growth under optimal conditions and eliminates seasonal variations. Moreover, the synthesis of nutritional and bioactive metabolites may be enhanced through elicitation approaches that stimulate stress responses to activate specific metabolic pathways to produce desired compounds. Such methods may include biotic (microorganisms) and abiotic (temperature, light, chemicals such as jasmonic acid and sodium chloride-NaCl) elicitors (Chandran et al. 2020; Ozyigit et al. 2023). For instance, excessive sodium chloride (NaCl) stress can be detrimental to plants, hindering growth and potentially leading to plant death. However, when NaCl concentrations are moderate, they can promote growth, increase the accumulation of secondary metabolites, and enhance pro-health components, antioxidants, and nutritional quality (Acosta-Motos et al. 2017; Chiappero et al. 2021). NaCl elicitation has significantly increased the levels of total phenolics, flavonoids, anthocyanins, and phenolic acids in *Melissa officinalis* L. cultures (Hawrylak-Nowak et al. 2021). It also triggers stress markers (H_2O_2, catalase, and peroxidase), activates phenylpropanoid enzymes (phenylalanine ammonia-lyase and tyrosine ammonia-lyase), and enhances phenolic content in common bean sprouts (Ampofo and Ngadi 2021). Furthermore, it has been found to improve saponin production in *Agave salmiana* plants cultured in vitro (Puente-Garza et al. 2021).

Additionally, in recent years, salinity has emerged as a significant environmental challenge, affecting agricultural productivity and ecological balance (Egea et al. 2023), and posing a significant threat to plant crops, especially in regions with limited freshwater resources (Tessema et al. 2023). Addressing this issue is crucial for ensuring global food security and maintaining ecological stability in areas affected by salinity. Understanding plant stress response and resilience is key to adapting agricultural practices in these environments while preserving the desired nutritional and medicinal benefits (Chele et al. 2021; Mukhopadhyay et al. 2021).

Inula crithmoides L., commonly known as golden samphire, is a flowering plant of the Asteraceae family. It thrives in salt marshes, maritime cliffs, and rocky coastal areas along the Mediterranean coast, Britain, and Western Asia (Clapham, 1962; eHALOPH 2023). Notably, golden samphire is both edible and aromatic. Its young leaves or shoots are suitable for consumption whether raw, cooked, or pickled (Zurayk and Baalbaki 1996; D'Agostino et al., 2022). Furthermore, many *Inula* species, including *I. crithmoides*, possess significant therapeutic effects, such as antimicrobial (Deriu et al. 2008), anti-inflammatory (Hernández et al. 2007), antioxidant (Kogure et al. 2004), and antihepatotoxic activities (Saygi et al. 2003), attributed to pharmacologically active compounds like polyphenols, including numerous quinic acid derivatives such as chlorogenic acid (Trendafilova et al. 2020). Moreover, plant in vitro culture has proven to be a valuable platform for this species' rapid and enhanced multiplication, providing a nursery for commercial cultivation systems (Rodrigues et al. 2023). Due to its therapeutic, nutritional, and aromatic properties, as well as its salt tolerance, golden samphire is considered a promising candidate for saline agriculture as a source of food and medicinal crops (Zurayk and Baalbaki 1996). Therefore, this study aimed to investigate the potential of using sodium chloride (NaCl) to enhance the accumulation of primary and secondary metabolites in *Inula crithmoides* L. shoots, as well as to evaluate its influence on their overall growth and oxidative stress responses aiming at increasing its nutritional and medicinal value.

Materials and methods

In vitro culture conditions and growth

Golden samphire plants were propagated in vitro using the micropropagation protocol outlined by Rodrigues et al. (2023). The shoots obtained were then cultivated in a basal MS medium supplemented with 0, 50, 100, and 200 mM NaCl. The medium, which contained 2% sucrose and 0.8% agar, was adjusted to a pH of 5.7–5.8 and autoclaved at 121 °C for 20 min. Cultures were maintained at a temperature of 25 ± 2 °C, with a 16/8 h light/dark photoperiod provided by LED light (2700 K) at an intensity of 3000 lx. The cultures were maintained for 6 weeks, and the number of shoots was counted. Additionally, the height of the tallest shoot and the length of the roots were measured.

Physiological parameters

Photosynthetic pigment determination

Photosynthetic pigments (total chlorophylls and carotenoids) were extracted by macerating 25 mg of fresh plant material with 4 mL of pure methanol, in triplicate. The optical density was measured at 470 nm, 653 nm, 666 nm, and 750 nm (Lichtenthaler 1987).

Hydrogen peroxide (H_2O_2) content

The hydrogen peroxide content was determined using the protocol developed by Loreto and Velikova (2001), which was adapted for use with 96-well plates. Fresh plant material (100 mg) was homogenised with 1 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged for 15 min at 12,000 x g. Then, 50 µL of the supernatant was mixed with 50 µL of 10 mM potassium phosphate buffer and 100 µL of

1 M potassium iodide. After 30 min in the dark, the absorbance was measured at 390 nm. The results were calculated by comparing them with an H_2O_2 calibration curve and expressed as micromoles of equivalent H_2O_2 per gram of fresh weight (µmol/g FW).

Primary metabolism

Extraction

Fresh plant material (250 mg) was extracted three times with 500 µL of 80% ethanol (v/v) at 80 °C for 30 min (Martins et al. 2011).

Proline content

Extracts (500 µL) were mixed with 1% (w/v) ninhydrin reagent prepared in 60% (v/v) acetic acid, incubated for 1 h at 100 °C, and the absorbance was measured at 520 nm. Results were calculated using a proline calibration curve and expressed as micromoles of proline equivalents per gram of fresh weight (µmol/g FW).

Total soluble proteins

The extracts (5 µL) were mixed with 250 µL of Bradford reagent and incubated for 30 min at room temperature. The absorbance was measured at 595 nm, and the results were calculated using a bovine serum albumin (BSA) calibration curve. The results are expressed as milligrams of BSA equivalents per gram of fresh weight (mg/g FW).

Total soluble sugars

The extracts (100 µL) were mixed with 100 µL of distilled water, 200 µL of 9% (w/v) phenol, and 1 mL of 96% sulfuric acid (Abid et al. 2021). After incubating at room temperature for 30 min, the absorbance was measured at 490 nm. Glucose was used as a standard, and the results were expressed as milligrams of glucose equivalent per gram of fresh weight (mg/g FW).

Secondary metabolism

Shikimic acid content

Fresh plant material (100 mg) was macerated with 2 mL of 0.25 M HCl and then centrifuged at 20,000 g for 15 min at 4 °C. Fifty microliters of supernatant were mixed with 500 µL of 1% (w/v) periodic acid. After a 3-hour incubation at room temperature, 500 µL of 1 M NaOH and 300 µL of 0.1 M freshly prepared glycine were added, and the absorbance was measured at 380 nm (Alzandi and Naguib 2020). Results were calculated using a shikimic acid calibration curve and expressed as milligrams of shikimic acid equivalents per gram of fresh weight (mg/g FW).

Phenylalanine ammonia-lyase (PAL) activity

Fresh plant material (50 mg) was homogenized in 1 mL of 0.1 M sodium borate buffer (pH 8.8) and centrifuged at 20,000 x g for 20 min at 4 °C. In a 96-well plate, 10 µL of the supernatant was mixed with 50 µL of L-phenylalanine (10 mM) and 140 µL of 0.1 M sodium borate buffer (pH 8.8). The mixture was incubated at 37 °C for 1 h, and the absorbance was measured at 290 nm. Enzyme activity was determined by comparing it with a calibration curve for cinnamic acids and expressed as micromoles of cinnamic acid per hour per gram of fresh weight (µmol/h/g FW).

Total phenolics, flavonoids, and hydroxycinnamic acids

Fresh plant material was extracted following the reported method for the analysis of primary metabolites. The total contents of phenolics (TPC), flavonoids (TFC) and hydroxycinnamic acids (THA) of the extracts were determined in 96-well plates, as previously detailed in Rodrigues et al. (2015). The TPC was determined according to the method described by Velioglu et al. (1998) by mixing 5 µL of the extracts with 100 µL of the Folin-Ciocalteu 10-fold diluted solution and 100 µL of 75 g /L of sodium carbonate, incubated for 90 min and read at 725 nm. TFC were estimated using the aluminium chloride ($AlCl_3$) colorimetric method described by Pirbalouti et al. (2013), modified to 96-well plates. For that, 50 µL of the extracts were mixed with 50 µL of a 2% $AlCl_3$-ethanol solution, incubated for 10 min and read at 420 nm. The THA was estimated using the hydrochloric acid (HCl)-ethanol method (Mazza et al. 1999) adapted to 96-well plates by Rodrigues et al. (2015) by mixing 20 µL of the extracts with 20 µL of 95% ethanol containing 0.1% HCl, and with 2% HCl, and read at 320 nm. Results for TPC, TFC and THA were correspondingly expressed as equivalents of gallic acid (GAE), rutin (RE), and caffeic acid (CAE) in milligrams per gram of fresh weight (FW), using calibration curves with standard solutions at concentrations ranging from 0.002 to 2 mg/mL.

High-performance liquid chromatography (HPLC) analysis

The extracts at a concentration of 10 mg/mL in a mixture of 90% ultrapure water and 10% methanol were analysed by HPLC-DAD (Agilent 1260 Infinity II Series LC system, Germany), which consisted of the following modules: 1260 quaternary pump (G7111B), 1260 vial sampler (G7129A),

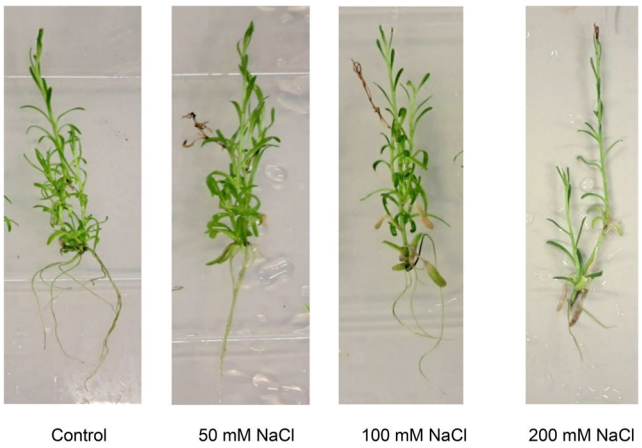

Fig. 1 General aspect of NaCl-elicited golden samphire shoots and roots, in comparison to the control, after 6 weeks of culture

and the 1260 diode array detector (G7115A). The data acquisition and instrumental control were performed using the OpenLab CDS software (version 2.6, Agilent Technologies). Analyses were performed using a Kinetex C18 column, 15 × 0.46 cm, with a 5 µm particle size (Phenomenex, USA). The mobile phase consists of a mixture of methanol (solvent A) and a 2.5% acetic acid aqueous solution with the following gradient: 0–5 min: 10% A, 5–10 min: 10–30% A, 10–40 min: 30–90% A, 40–45 min: 90% A, 45–55 min: 90 – 10% A, and 55–60 min: 10% A, using a flow rate of 0.5 mL/min. The injection volume was 20 µL at a draw speed of 200 µL/min. The detector was set at 255, 280, 320, and 350 nm. For identification purposes, the retention parameters of each assay were compared with the standard controls, and the peak purity was assessed using UV-visible spectral reference data. The levels of the various compounds were extrapolated from calibration standard curves. Commercial standards of gentisic, 4-hydroxybenzoic, chlorogenic, caffeic, vanillic, coumaric, cinnamic, and ferulic acids, luteolin-7-O-glucoside, naringenin-7-O-glucoside, rutin, naringenin, chrysin, and flavone were dissolved in ethanol (1000 mg/L) and then diluted with ultrapure water to the desired concentration.

Statistical analysis

The results were expressed as the mean ± standard deviation (SE), and each experiment was conducted at least three times. Significant differences were assessed using ANOVA followed by Duncan's multiple range test (DMRT). P values less than 0.05 were considered significant. All statistical analyses were conducted using the XLSTAT statistical package in Microsoft Excel (version 2013, Microsoft Corporation).

Results

Growth and physiological parameters

Different concentrations of NaCl did not have a significant effect on the growth of aerial parts, as indicated by a similar number of shoots (1.67–2.29 cm) and height (8.79–10.67 cm) across all treatments (Figs. 1 and 2). However, the presence of NaCl in the medium significantly reduced the root length from 8.47 cm in the control plants (without NaCl) to 3.50 to 4.73 cm (50 to 200 mM NaCl) (Fig. 2). Furthermore, the overall moisture content of the plant has been observed to decrease from 94.1–94.5–91.4% when salinity exceeds 200 mM NaCl (data not shown). Interestingly, the total chlorophyll and carotenoid contents increased with the rising NaCl concentration in the media, following

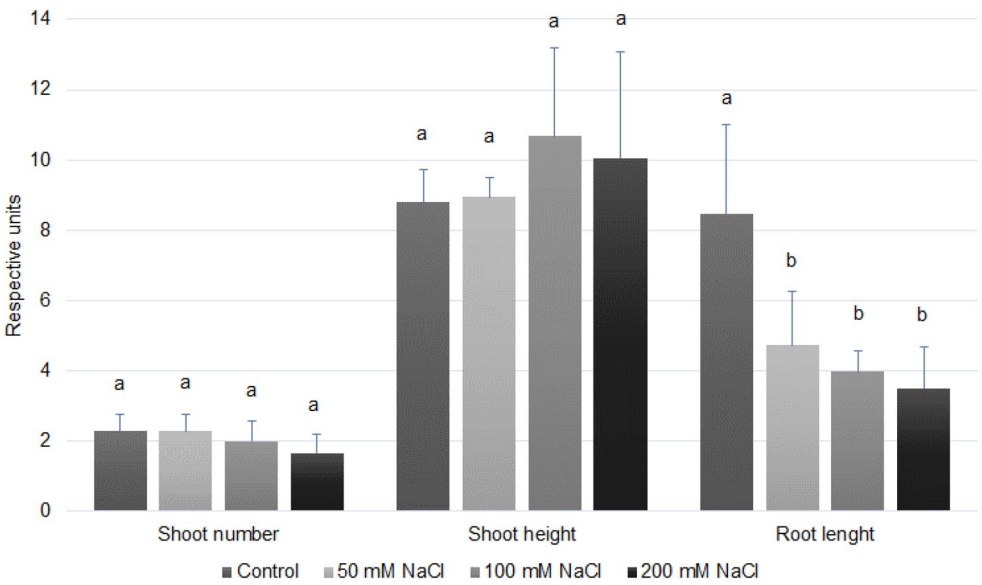

Fig. 2 Effect of exposure to various concentrations of NaCl (50, 100, and 200 mM) on the shoot number, maximum shoot height, and root length of in vitro cultures of *I. crithmoides*. For each assay, the results were analysed using a one-way analysis of variance (ANOVA) and the bars on the graph bars labelled with different letters (a–d) are significantly different at $p < 0.05$ (Duncan's test)

a dose-dependent trend. For instance, plants grown under controlled conditions (without salts) had 0.26 mg/g and 0.04 mg/g of total chlorophylls and carotenoids, respectively. However, with an increase in salinity to 50 and 100 mM NaCl, the levels correspondingly rose to 0.40–0.42 mg/g of FW and 0.6–0.7 mg/g of FW. When exposed to 200 mM, the golden samphire plants exhibited the highest levels of chlorophylls (0.72 mg/g FW) and carotenoids (0.10 mg/g FW) (Fig. 3A). Conversely, an opposite trend was observed for H_2O_2 levels, with its concentration decreasing as salinity increased. Plants grown without salts (control) had 5.71 µmol/g FW of H_2O_2, which decreased to 4.45–4.51 µmol/g FW when the golden samphire was grown at 50 and 100 mM, while the lowest levels were observed under the NaCl 200 mM condition (1.75 µmol/g FW) (Fig. 3B).

Primary metabolism

Higher contents of both total soluble proteins and sugars were observed in salt-free conditions (0.23 and 3.72 mg/g FW, respectively) and in plants growing in a saline medium. However, no significant differences were observed between the different concentrations of NaCl (proteins: 0.68–0.73 mg/g FW; sugars: 3.86–3.87 mg/g FW) (Fig. 4). In

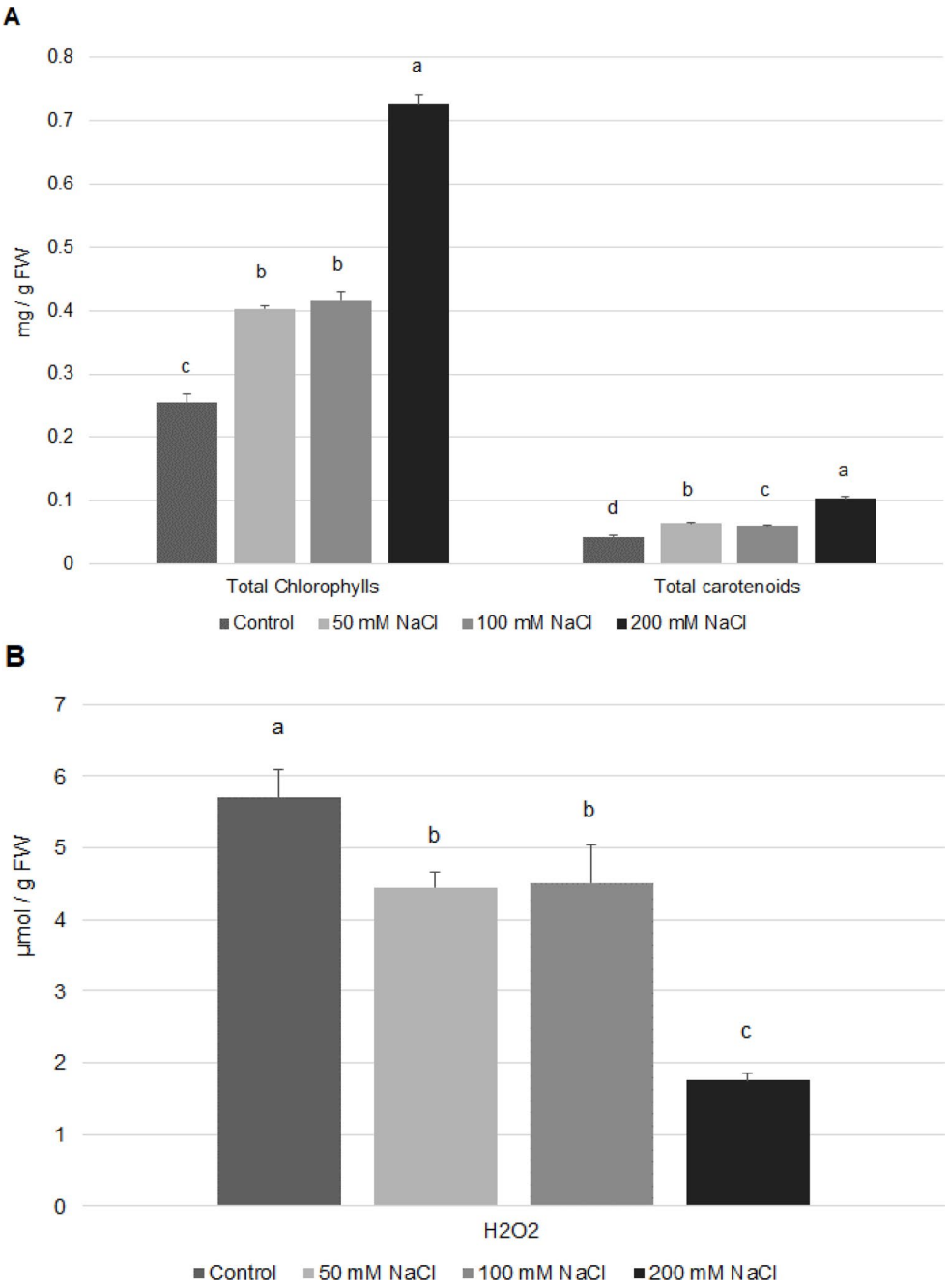

Fig. 3 Effect of exposure to various concentrations of NaCl (50, 100, and 200 mM) on pigments (total chlorophylls and carotenoids) (**A**) in in vitro cultures of *I. crithmoides*. For each assay, the results were analysed by one-way analysis of variance (ANOVA) and the graph bars followed by different letters (**a**–**d**) are significantly different at $p < 0.05$ (Duncan's test)

turn, there was a fivefold increase in proline content when plants were cultured under 200 mM NaCl conditions (8.49 µmol/g FW) compared to control plants (1.62 µmol/g FW) (Fig. 4).

Secondary metabolism

The shikimic acid content showed a significant increase from plants grown without salt and 50 mM NaCl (0.11–0.14 mg/g of FW) to plants grown under 100 mM NaCl or more (0.22 mg/g of FW) (Fig. 5A). In turn, PAL activity increased 11-fold in plants exposed to 200 mM NaCl (15.9 µmol/h/g FW) compared to the control (1.40 µmol/h/g FW) (Fig. 5B), along with elevated levels of phenolics, flavonoids, and hydroxycinnamic acids (0.54, 0.95, and 4.16 mg/g of FW, respectively) (Fig. 5C).

The HPLC-DAD analysis of phenolic compounds in golden samphire plants exposed different varying concentrations of NaCl (50, 100, and 200 compared comparison to the revealed distinct trends in the levels of these (Table 1 and Fig. S1). The phenolic compounds in golden samphire plants exhibit distinct variations in response to NaCl exposure, with some compounds increasing, others remaining stable, and some decreasing in concentration. For example, 4-hydroxybenzoic acid, the most abundant compound, exhibited an increasing concentration as the NaCl concentration increased. It reached its maximum at 200 mM NaCl with 0.29 mg/g of DW, compared to 0.08 mg/g of DW in the control. Chlorogenic acid, on the other hand, reached its highest concentration at 100 mM NaCl (0.31 mg/g DW), but decreased to 0.18 mg/g DW at 200 mM NaCl, showing a significant deviation from the control levels (< 0.01 mg/g DW). In contrast, naringenin-7-O-glucoside, luteolin-7-O-glucoside, and gentisic acid reached their peak at 50 mM NaCl (0.19, 0.07, and 0.06 mg/g DW, respectively), followed by a slight decrease at higher NaCl concentrations. Conversely, the rutin and chrysin content declined with higher NaCl concentration, reaching the lowest levels at 200 mM NaCl (0.02 and 0.01 mg/g DW, respectively). The concentration of naringenin decreased under salt stress compared to control levels (0.05 mg/g of DW), with no variation observed between the different NaCl concentrations (0.03 mg/g of DW). Flavone, vanillic, coumaric, cinnamic, caffeic, and ferulic acids exhibited modest fluctuations and generally maintained relatively low levels (0.01–0.02 mg/g DW).

Discussion

Growth and physiological parameters

Elevated soil salinity typically has a negative impact on plant growth by increasing osmotic stress on roots, inhibiting water absorption, disrupting nutrient uptake, and ultimately leading to reduced biomass and impaired overall plant health (Balasubramaniam et al. 2023). However, the golden samphire did not experience a significant impact on the growth of its aboveground parts up to 200 mM NaCl. Other halophyte species, such as *Atriplex halimus*, have

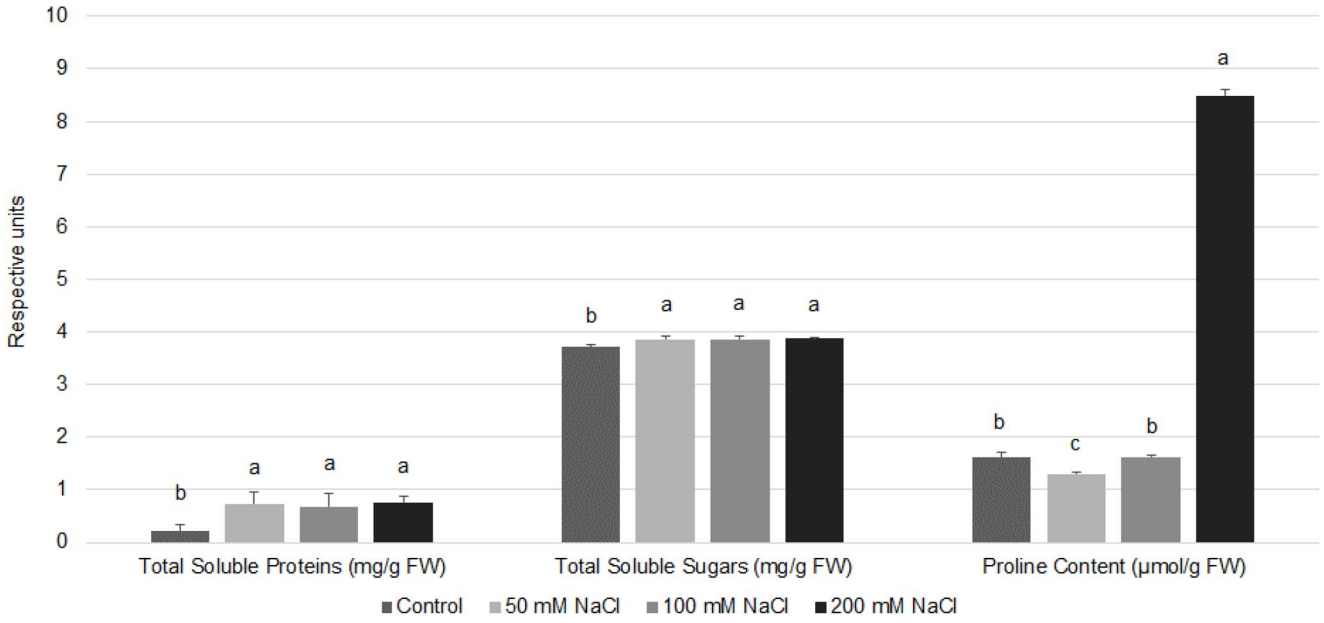

Fig. 4 Effect of exposure to various concentrations of NaCl (50, 100, and 200 mM) on primary metabolites (total soluble sugars, soluble proteins, and proline contents) in in vitro cultures of *I. crithmoides*. For each assay, the results were analysed using a one-way analysis of variance (ANOVA) and the bars on the graph bars labelled with different letters (a–c) are significantly different at $p < 0.05$ (Duncan's test)

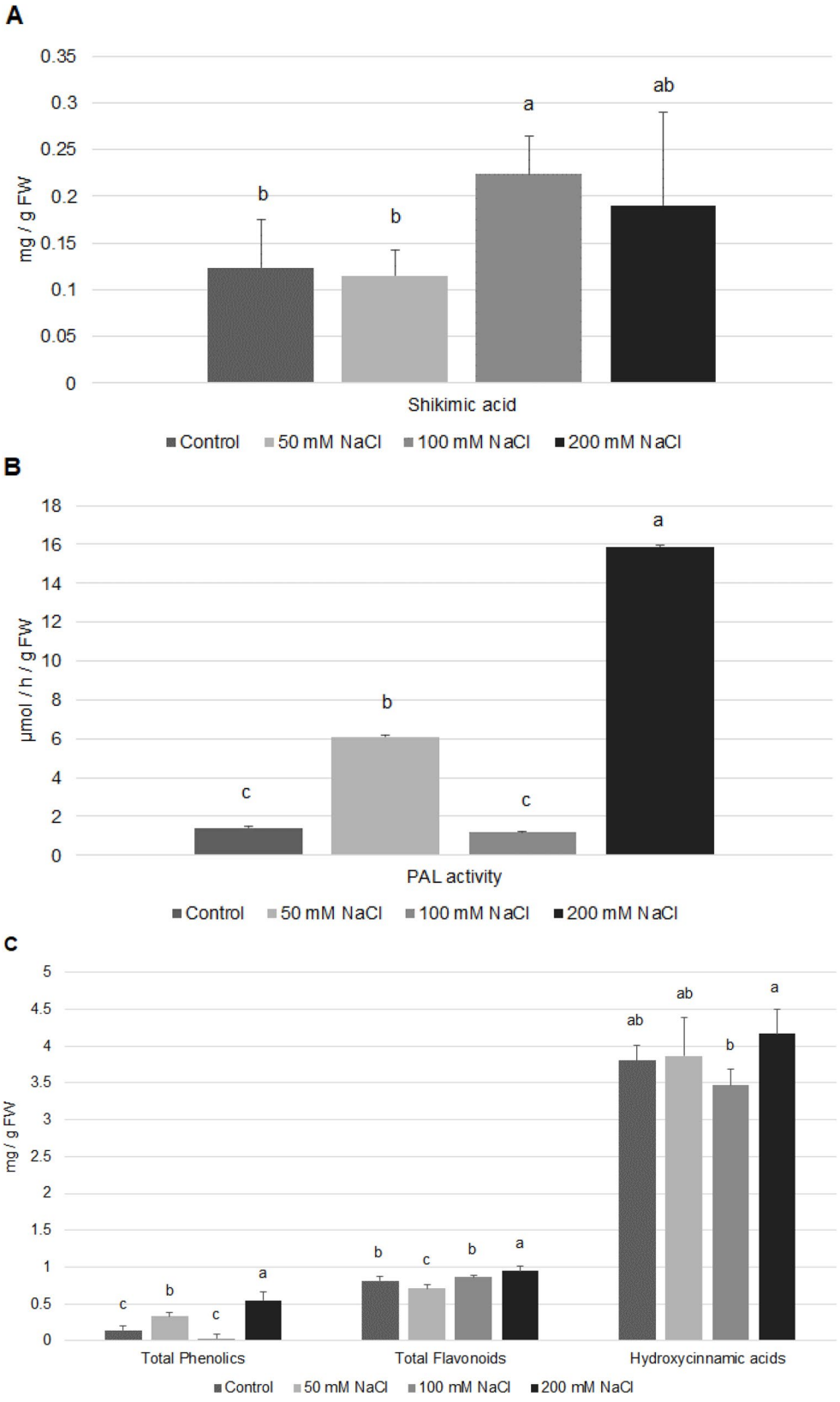

Fig. 5 Effect of exposure to various concentrations of NaCl (50, 100, and 200 mM) on the secondary metabolism, including shikimic acid (**A**), PAL activity (**B**), and the total content of phenolics, flavonoids, and hydroxycinnamic acids (**C**), in in vitro cultures of *I. crithmoides*. For each assay, the results were analysed using a one-way analysis of variance (ANOVA) and the graph bars followed by different letters (**a**–**c**) are significantly different at $p < 0.05$ (Duncan's test)

Table 1 Qualitative and quantitative (mg/g DW) analysis of phenolic compounds in golden samphire plants exposed to different concentrations of NaCl (50, 100, and 200 mM) using HPLC-DAD. For each compound, the results were analysed using a one-way analysis of variance (ANOVA), and the values followed by different letters (a–d) are significantly different at $p < 0.05$ (Duncan's test)

Compound*	Rt** (min)	Control	50 mM NaCl	100 mM NaCl	200 mM
Gentisic acid	11.4	< 0.01	0.06 ± 0.002 [a]	Nd	0.04 ± 0.001 [b]
4-Hydroxybenzoic acid	11.7	0.08 ± 0.004 [c]	0.21 ± 0.01 [b]	0.23 ± 0.01 [b]	0.29 ± 0.02 [a]
Chlorogenic acid	12.7	< 0.01	0.31 ± 0.02 [a]	0.12 ± 0.007 [c]	0.18 ± 0.01 [b]
Caffeic acid	13.8	< 0.01	< 0.01	< 0.01	< 0.01
Vanillic acid	13.9	0.02 ± 0.001 [a]	0.01 ± 0.002 [b]	Nd	0.01 ± 0.001 [b]
Coumaric acid	17.2	< 0.01	0.01 ± 0.001 [b]	Nd	0.02 ± 0.001 [a]
Ferulic acid	18.2	0.01 ± 0.001 [a]	0.01 ± 0.001 [a]	Nd	0.01 ± 0.001 [a]
Luteolin-7-O-glucoside	19.8	0.07 ± 0.008 [b]	0.17 ± 0.02 [a]	0.07 ± 0.009 [b]	0.09 ± 0.01 [b]
Naringenin-7-O-glucoside	19.9	0.03 ± 0.001 [d]	0.19 ± 0.002 [a]	0.05 ± 0.001 [c]	0.07 ± 0.001 [b]
Rutin	20.4	0.04 ± 0.001 [a]	0.03 ± 0.001 [b]	0.03 ± 0.001 [b]	0.02 ± 0.001 [c]
Cinnamic acid	26.0	< 0.01	< 0.01	0.01 ± 0.001 [a]	0.01 ± 0.001 [a]
Naringenin	26.2	0.05 ± 0.003 [a]	0.03 ± 0.002 [b]	0.03 ± 0.002 [b]	0.03 ± 0.002 [b]
Chrysin	34.4	0.03 ± 0.001 [a]	0.02 ± 0.001 [b]	0.02 ± 0.001 [b]	0.01 ± 0.001 [c]
Flavone	34.6	0.01 ± 0.001 [a]	0.01 ± 0.006 [a]	< 0.01	< 0.01

*Identification was based on comparing the retention parameters with the standard controls and accessing the peak purity using UV-vis spectral reference data. Nd: Below HPLC-DAD sensitivity

**Retention time

shown an increase or unchanged leaf fresh weight at moderate salinities (0–200 mM NaCl) (Bendaly et al. 2016), as well as *Arthrocnemum indicum*, which exhibited unchanged shoot height up to 300 mM NaCl (Nisar et al. 2021). Furthermore, the salinity hindered the growth of golden samphire roots. In fact, roots are the primary organ involved in the uptake of water and nutrients before they are transported to the aboveground parts. Therefore, roots are the first organ to sense and respond to salt stress, enabling the plant to quickly adapt and remain functional by altering the root anatomy. This can include the formation of apoplastic barriers, which are modifications of the cell wall that help prevent the entry of toxic ions through the root apex, while also regulating the flow of Na^+ to the shoots (Shahid et al. 2020). This strategy may explain the reduced root growth and the lack of impact on golden samphire shoots.

The average chlorophyll content of the golden samphire plants increased with increasing salinity, indicating that an increase in chlorophyll and carotenoid content appears to contribute to the salt resilience of golden samphire. An increase in photosynthesis can lead to higher growth rates and biomass, provided that the carbon source is not limited. This can explain the consistent plant growth observed in golden samphire in vitro cultures (Maschler et al. 2022). Moreover, similar to the current study, two halophyte species from the Juncaceae family (*J. acutus* and *J. maritimus*) have shown similar or increased pigment contents (chlorophylls and carotenoids) when grown in moderate salinities (100–200 mM NaCl) compared to plants irrigated with salt-free nutrient solution (Al Hassan et al. 2017). A similar trend was also observed in the halophyte grass *Aeluropus littoralis* under 600 mM NaCl (Hashemipetroudi et al. 2022), as well as in *Limonium santapolense, L. virgatum, L. girardianum*, and *L. narbonense* cultivated at moderate salinities (0–400 mM) (Hassan et al., 2017).

Salt stress usually induces the accumulation of Reactive Oxygen Species (ROS), such as hydrogen peroxide (H_2O_2), which can lead to increased oxidative damage to membrane lipids, proteins, and nucleic acids, causing irreversible metabolic dysfunction (Balasubramaniam et al. 2023). In this study, the concentration of H_2O_2 in golden samphire plants decreased as the salinity of the growing media increased, which is consistent with findings for several other halophytes, including *Chrithmum maritimum* (Hamdani et al. 2017), *Atriplex halimus, Nitraria retusa* (Boughalleb et al. 2010), *Prosopis strombulifera* (Reginato et al. 2019), and *Suaeda salsa* (Cai-Hong et al. 2005). It is known that the plant's capacity to neutralize ROS is directly associated with the levels and performance of antioxidant enzymes, such as catalase, peroxiredoxin, glutathione peroxidase-like enzymes, and ascorbate peroxidase (Smirnoff and Arnaud 2019). When these mechanisms are present, it has been shown that NaCl does not significantly alter the levels of H_2O_2 due to the increased activity of APX (Tzortzakis et al. 2020). Moreover, the levels of osmoprotectants such as proline, and secondary metabolites like phenolic compounds may also contribute to decreasing the level of ROS (Kebert et al. 2022).

Overall, the NaCl treatment reveals the remarkable resilience of golden samphire, demonstrating its ability to sustain growth and physiological functions such as photosynthesis and management of oxidative stress under moderate salinity levels.

Primary metabolism

Primary metabolism refers to the fundamental biochemical processes that are essential for plant growth, development, and survival, such as sugars, proteins, and lipids (Reshi et al. 2023). Under saline conditions, plants often respond by accumulating organic solutes such as proteins, proline, soluble sugars, glycine betaine, and polyols. These small soluble compounds act as osmoprotectants, helping with intracellular osmotic adjustment and detoxification of ROS (Slama et al. 2015; dos Santos et al. 2022). For example, the accumulation of proline protects and stabilizes ROS-scavenging enzymes, activates alternative detoxification pathways, and enhances the activity of methylglyoxal detoxification enzymes, peroxidase, glutathione S-transferase, superoxide dismutase, and catalase. It also increases the glutathione redox state under salt stress (Shuyskaya et al. 2020). Interestingly, they accomplish these protective functions without negatively impacting cellular metabolism while safeguarding the integrity of the cell membrane (Slama et al. 2015). The levels of total proteins, soluble sugars, and notably proline have increased in golden samphire in vitro cultures when exposed to salinity. A trend already reported in *Inula* species grown in an environmental chamber (Pehlivan and Guler, 2019), as well as common to many other salt-tolerant plants subjected to a wide range of salinities (0–750 mM NaCl), including, for example, *Frankenia pulverulenta, Atriplex prostrata* (Bueno et al. 2020), *Chrithmum maritimum* (Hamdani et al. 2017), *Aeluropus littoralis* (Hashemipetroudi et al. 2022), or *Lycium humile* (Palchetti et al. 2021).

In addition, the increased soluble sugar and protein content in plants, as a result of their adaptive responses to environmental stresses such as NaCl elicitation, can directly benefit human health when these plants are consumed. Soluble sugars such as glucose, fructose, and sucrose are essential energy sources in human nutrition and also play significant roles in carbohydrate metabolism. The increased presence of these sugars in plants not only reflects their stress adaptation mechanisms but also potentially enhances the nutritional value of the plants, providing a richer energy source for human consumption (Prinz 2019). Soluble proteins, including proline, play vital roles in supporting immune function and providing energy for collagen synthesis, essential for wound healing. Proline, in particular, supports gut health maintenance, contributes to antimicrobial peptides, and aids in skin barrier function, highlighting its critical role in innate immunity and metabolic regulation. These diverse roles underscore the importance of dietary proteins and amino acids in maintaining health and supporting physiological processes (Patriarca et al. 2021; Hepworth 2023).

Secondary metabolism

Secondary metabolism involves the synthesis of a diverse range of specialized compounds, such as polyphenols, that play a role in the defence of plants against environmental stress conditions (dos Santos et al. 2022). These compounds may also play a role in signalling pathways that regulate stress responses and cellular homeostasis, enhancing the adaptability of a plant and exhibiting potent antioxidant properties, essential for eliminating reactive oxygen species (ROS) such as H_2O_2 (Reshi et al. 2023). The shikimic acid pathway is essential for the biosynthesis of phenolic compounds via the phenylpropanoid pathway, with phenylalanine serving as the primary substrate for their synthesis. This process is facilitated by an essential enzyme called phenylalanine-ammonia-lyase (PAL), which is crucial for phenolic biosynthesis (Reshi et al. 2023). In this study, the golden samphire plants exhibited higher levels of shikimic acid above 100 mM NaCl. Additionally, they showed increased PAL activity and higher levels of phenolics, flavonols, and hydroxycinnamic acids at maximum salinity (200 mM NaCl). The same trend has been previously observed in *I. chrithmoides* cultivated in a greenhouse under different levels of NaCl (0–600 mM) (Chaura et al. 2015; Al Hassan et al. 2016), as well as in other halophyte species exposed to salt stress conditions (0–600 mM NaCl), such as *Frankenia pulverulenta* (Bueno et al. 2020), *Juncus maritimus, J. acutus*, and *J. articulatus* (Al Hassan et al. 2017b).

Furthermore, phenolic acids, such as 4-hydroxybenzoic and chlorogenic acids, were the compounds most affected by NaCl elicitation. These acids have been described as crucial signalling molecules in plants' adaptive responses to abiotic stress, especially salinity. These compounds play a multifaceted role in detoxifying harmful substances, protecting plants against oxidative damage, and increasing antioxidant capacity. As a result, they mitigate the detrimental effects of ROS on plant metabolism by enhancing photosynthetic activity (Soviguidi et al., 2021). The increased production of secondary antioxidant metabolites in golden samphire under salinity treatments is likely to contribute to the observed rise in chlorophyll and carotenoid levels, as well as the significant reduction of H_2O_2. This suggests a crucial role for these compounds in reducing ROS, thereby improving photosynthetic efficiency.

Additionally, phenolic acids and flavonoids, which are key secondary metabolites that are particularly increased by NaCl elicitation in golden samphire shoots, are known for various medicinal benefits. For example, gentisic acid has been linked to several health-promoting effects, such as antioxidant, anti-inflammatory, hepato- and neuroprotective, and antimicrobial activities (Abedi et al. 2020). Chlorogenic acid, a compound commonly found in *Inula* species, has

also attracted attention for its wide range of pharmacological effects, including neutralizing oxygen radicals, boosting superoxide dismutase concentrations, and moderating the synthesis and secretion of inflammatory mediators. Moreover, it has demonstrated antibacterial and antiviral properties, along with its potential role in managing blood sugar and lipid levels, which could be beneficial for treating and preventing chronic diseases such as cardiovascular diseases and diabetes (Miao and Xiang 2020). Similarly, 4-hydroxybenzoic acid exhibits antimicrobial, antialgal, antimutagenic, antiestrogenic, hypoglycemic, and anti-inflammatory properties. It also has anti-platelet aggregation, nematicide, antiviral, and antioxidant effects. It is also commonly used as a preservative in various products, including cosmetics, pharmaceuticals, food items, and beverages (Manuja et al. 2013). Additionally, the flavonoid derivatives luteolin-7-O-glucoside and naringenin-7-O-glucoside also offer a multitude of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, antiviral, neuroprotective, and cardioprotective properties (Ullah et al. 2020). The heightened concentration of bioactive compounds such as gentisic acid, chlorogenic acid, 4-hydroxybenzoic acid, luteolin-7-O-glucoside, and naringenin-7-O-glucoside in NaCl-treated golden samphire shoots indicates that NaCl elicitation may substantially boost the medicinal properties of golden samphire, potentially elevating its efficacy as a natural remedy for various health conditions.

Conclusions

The investigation into NaCl elicitation's impact on golden samphire demonstrated a significant enhancement in the accumulation of both primary and secondary metabolites within in vitro shoot cultures. This process notably augmented levels of soluble sugars, proteins (especially proline), and a range of bioactive phenolic compounds such as gentisic acid, chlorogenic acid, 4-hydroxybenzoic acid, luteolin-7-O-glucoside, and naringenin-7-O-glucoside, without adversely affecting plant growth. Moreover, it improved physiological responses such as photosynthesis and oxidative stress resilience at moderate salinity levels. These outcomes elucidate NaCl elicitation's potential in elevating *I. crithmoides* shoots' nutritional and medicinal properties, highlighting its adaptability for saline agriculture and its promising utility in the health and nutrition sectors.

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Data availability Data will be made available on request.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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