Insight into phytohormonal modulation of defense mechanisms to salt excess in a halophyte and a glycophyte from Asteraceae family

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Abstract

Aims The aim of this study was to compare the efficiency of three defense mechanisms (ionic balance, osmotic adjustment and counteracting oxidative stress) under low, moderate and high soil salinity in two related species of contrasting tolerance to salinity: the halophyte *Aster tripolium* and the glycophyte *Aster alpinus*, and to elucidate their phytohormone-mediated regulation.

Methods The phytohormonal profiling was performed to assess correlations between the pool of plant growth regulators and parameters depicting ionic homeostasis, osmotic adjustment and antioxidant system.

Results Defense mechanisms in both species were based on accumulation/activity of distinct compounds (Na^+ and K^+ ions, antioxidants), but differences among species concerned particularly soluble carbohydrates and betaines. The halophyte accumulated mannitol, uronic acids and sucrose, while the glycophyte mostly glucose and proline-betaine. The halophyte responses also correlated with changes in the content of plant growth promoting PGRs, as well as jasmonates and

benzoic acid. The glycophyte responses corresponded with changes in content of abscisic acid and ethylene precursor, as well as salicylic acid.

Conclusions We provided evidence that benzoic acid rather than salicylic acid is involved in salt tolerance in the halophyte and elevated SA content may enhance vulnerability to salt excess. An important element of tolerance trait is also JA-GA network that influences the intensity of defense responses. This study uncovers new aspects of internal phytohormonal regulation of plant reaction to soil salinity and enables further insight into extremophyte biology.

Keywords Antioxidants · Extremophyte · Ions · Osmolytes · Phytohormones · Relative taxa · Salt excess

Abbreviations

ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
Aux	auxins
BA	benzoic acid
Car	carotenoids
CAT	catalase (EC 1.11.1.6)
Chl	chlorophylls
Cyt	cytokinins
DW	dry weight
EC	electrical conductivity
FV	total flavonoids
FW	fresh weight
GA	gibberellins
GlyB	glycine betaine
GTI	growth tolerance index

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JA	jasmonates
MDA	malondialdehyde
OPDA	oxophytodienoic acid
PGR	plant growth regulators
POD	peroxidase
Pro	proline
ProB	proline betaine
SA	salicylic acid
SL	stem length
TBARS	thiobarbituric acid-reactive-substances
TPC	total phenolic content
TSC	total soluble carbohydrates
WC	water content

Introduction

Excessive soil salinity is a destructive environmental factor that greatly reduces plant growth and productivity. General plant tolerance to suboptimal salinity mostly depends on the activation of a series of conserved mechanisms, among which three are particularly important: control of ion homeostasis, accumulation of osmolytes to maintain cellular osmotic balance, and activation of antioxidant system to counteract oxidative stress (Flowers and Colmer 2015; Rozema and Schat 2013). These basic mechanisms are not specific for stress-tolerant species only but are shared by all plants, and tolerance range in various species is attributed to their relative efficiency (Bartels and Dinakar 2013). Notwithstanding, defense responses and strategies to survive and sustain growth are highly complex and involve multiple pathways and molecules. Due to such complexity, it is often problematic to obtain a complete understanding of how a particular species responds to salinity (Li et al. 2010).

Recently, extended knowledge has been gathered considering physiological performance of halophytes in saline areas. These plants are extremely salt tolerant – they usually grow and survive in environments with salt concentrations as high as 5 g l^{-1} (Joshi et al. 2015), being capable of surviving under a salt shock, for example due to tidal or rainfall events. Their structure and metabolism are notably adapted to suboptimal growth conditions of saline habitats (Ali and Yun 2017). Halophytes respond to salinity at cellular, tissue and whole plant level (Bercu et al. 2012; Kumari and Parida 2018). The main tolerance mechanism is based on ion

accumulation and sequestration, to balance an internal osmotic status with external salinity (Flowers et al. 2010). This mechanism is supported by synthesis of versatile compatible solutes and significant boost in antioxidant activity. Insight into physiological control of halophyte responses to excessive salinity is the key to plant engineering for salt tolerance (Kumari et al. 2015; Rozema and Schat 2013). Particularly, the regulatory network conditioning salt tolerance in these extremophytes needs clarification (Maršálová et al. 2016). An important aspect are the interplays between endogenous phytohormones (PGRs) and their contribution to activation of a particular defense strategy (Benjamin et al. 2019; Ellouzi et al. 2014).

In stress physiology, PGRs are characterized as cellular signal molecules involved in the regulation of plant responses to unfavorable environmental conditions (Peleg and Blumwald 2011). Their signaling pathways are linked in a complex network, which provides plants with a regulatory potential to directly adapt to stress factors and avoid serious growth deterioration (Llanes et al. 2018). Even slight variations in endogenous PGR contents may change their physiological role and phytohormonal balance of an entire organism (Pieterse et al. 2009). In recent years, knowledge on PGR role in halophyte responses has grown significantly, e.g. thanks to the studies on hormone-biosynthetic pathways in salt-sensitive glycophytic species (Gharbi et al. 2017; Gong et al. 2005; Prerostova et al. 2017). The wide range of salinity levels that halophytes can withstand is related to numerous biochemical responses involved in stress tolerance in these species. The most pronounced effects are various growth alterations, directly influenced by PGRs (Guo et al. 2020; Wiszniewska et al. 2019). Growth regulators control also the activation and efficiency of the general stress defense reactions (Julkowska and Testerink 2015; Sharma et al. 2019; Souza et al. 2017). Salinity leads to modifications in signal transduction of growth promoting as well as stress hormones, which may promote activation of specific protective mechanisms (Eyidogan et al. 2012). Although plant response to salt stress depends on many factors and their relative influence can vary between genotypes, still endogenous PGRs are considered critical in modulating physiological responses that eventually lead to adaptation to a harmful environment (Peleg and Blumwald 2011). Despite its uniformity and abundance in plants,

pathways of phytohormonal control may differ substantially between salt-tolerant and salt-sensitive genotypes (Dias et al. 2015; Prerostova et al. 2017).

Studies on related taxa of contrasting stress tolerance may facilitate understanding of the relative contribution of particular defense responses to plant ability to survive in stressful conditions (Al Hassan et al. 2017; Koźmińska et al. 2019). In this study, we have implemented such an approach to investigate the efficiency of salinity defense mechanisms and their correlations with fluctuation of endogenous PGRs in two species from Asteraceae family: a halophyte *Aster tripolium* (syn. *Tripolium pannonicum* subsp. *tripolium* (L.) Greuter), and a glycophyte *Aster alpinus* L. Sea aster (*A. tripolium*) is a perennial halophyte native to Eurasia and northern Africa, confined in its distribution to salt marshes, estuaries and occasionally to inland saline areas. This species grows up to 50 cm tall with fleshy lanceolate leaves and purple ray florets flowering from July to September. Sea aster is suitable for well-drained light (sandy), medium (loamy) and heavy (clay) soils, with wide range of pH. It cannot grow in the shade (Shennan et al. 1987; Duarte et al. 2015). *A. tripolium* is already identified and used commercially as food source (Ventura et al. 2013). Alpine aster (*A. alpinus*) is native to the mountains of Europe, including the Alps and Pyrenees, and is the only North American species of 180 ones in this genus (Pennisi 2001), cultivated worldwide as an ornamental plant. This species belongs to the ecological group of xeromesophytes. It is an herbaceous perennial plant forming a clump to 30 cm in height, with narrow dark green leaves and daisy-like flowerheads to 5 cm in width. It grows in moderately fertile, medium moisture, well-drained soils in full sun, in narrow pH range (6–7.5). This species can survive medium heat and requires at least 90 frost-free days for proper growth and may struggle in the hot and humid climate (Korolyuk 1997; Szeląg and Kobiv 2017; Filippova et al. 2019).

The species have been selected as a representatives of the complex genus, taking into account their distinct ecological features, mainly an ability to sustain growth in saline environment. *A. tripolium* is a halophyte capable of maintaining ionic homeostasis and counteract water loss. *A. alpinus*, as an alpine species, is also tolerant to limited water supply, however in its natural habitat it does not challenge osmotic disturbances caused by excessive uptake of soluble salts. For this

reason it can be chosen as a referential species of glycophytic character.

The aim of this study was to compare the efficiency of three general defense mechanisms under low, moderate and high salinity in the halophyte and the glycophyte, and to elucidate their phytohormone-mediated regulation. Our basic assumption was that the responses that are critical for tolerance should be more efficiently expressed in the halophyte than the glycophyte. This study also aimed at identification of particular PGRs related to species-dependent responses and the prevailing mode of defense mechanisms under distinct levels of salinity in salt-tolerant and salt-sensitive *Aster* species.

Materials and methods

Plant material

Seeds of *A. tripolium* (*Tripolium pannonicum* subsp. *tripolium* (L.) Greuter) were obtained from the Natural Park ‘La Albufera’ (Province of Valencia, Spain), obtained from the seed socks of the ‘Servicio Devesa-Albufera’, and seeds of *A. alpinus* were purchased from a commercial supplier (POLAN Spółka z o.o.). Formal identification of plant material was conducted by Dr. Ewa Sitek, PhD, from Department of Botany, Physiology and Plant Protection, University of Agriculture in Kraków, Poland. Voucher specimens have been deposited in herbarium of the Department.

Plant growth conditions and treatment

Germination was carried out on standard Petri dishes at 25 °C and 16 h photoperiod. After five weeks, seedlings were individually transplanted into 1 L pots ($\varnothing = 11$ cm), containing a moistened mixture of soil (50%), perlite (25%) and sand (25%). During the entire course of plant growth the substrate was kept moist by adding 200 ml Hoagland’s nutrient solution to each pot twice a week.

Salt treatments started three weeks after the seedling transplantation. To this end ten individual seedlings (ten biological replicates) of uniform size per species and per treatment were selected and transferred to the pots; ten pots of each set were treated for four weeks. The plants were watered twice a week with freshly prepared aqueous NaCl solutions of increasing concentration (200,

400 and 600 mM, representing and referred as to low, moderate and high salt stress, respectively), using 1.5 L per tray. The control, non-treated plants were watered with distilled water. All experiments were conducted in a controlled environment in a greenhouse, under the following conditions: long-day photoperiod (16 h of light), temperature of 23 °C during the day and 17 °C at night.

Soil analysis

The electrical conductivity ($EC_{1.5}$) of the substrate was checked at the end of the treatments (after 4 weeks) (Suppl. Table 1). Soil samples were taken from the five pots selected per treatment, air-dried and then passed through a 2 mm sieve. A soil: water (1:5) suspension was prepared in distilled water and stirred for 1 h at 600 rpm and 21 °C. EC was measured with a Crison Conductivity meter 522 and expressed in dSm^{-1} . The increase in $EC_{1.5}$ was basically linear, correlating with the concentration of the saline solutions used in the treatments. Substrate conditions varied under low salinity (9.05 dSm^{-1} for *A. alpinus* and 7.11 dSm^{-1} for *A. tripolium*, $P < 0.05$), whereas under moderate and high salinity they were the same for both the halophyte and the glycophyte (Suppl. Table 1).

Plant growth parameters

After four weeks of salt treatment the following growth parameters were determined: stem length (SL), fresh (FW) and dry weight (DW), and percentage of water content in the leaves. SL and DW were expressed as percentages of the mean values determined for the corresponding controls, taken as 100%. Control values of SL and DW were as follows:

41 cm and 0.29 g (*A. tripolium*) and 21 cm and 0.22 g (*A. alpinus*), respectively.

To determine water content (WC), leaf samples were weighed (FW), dried at 65 °C until constant weight (72 h) and then reweighed (DW); water content of each sample was calculated as: $WC\% = [(FW - DW) / FW] \times 100$.

Growth Tolerance Index (GTI) (%) was calculated on the basis of DW of shoots using the formula:

$GTI = (\text{mean DW of salinity-treated plants} / \text{mean DW of untreated plants}) \times 100$.

Ion content determination

Dry plant material was digested in a closed microwave system using HNO_3 , and the contents of Na and K were assessed using a PerkinElmer atomic emission spectrometer ICP-OES Optima 7300 DV and multi-element ICP-IV Merck standard solution. The accuracy of the analytical methods was verified using GSS-8 certified reference material (GBW 07408; State Bureau of Meteorology, Beijing, China).

The content of chloride in plant samples was determined in aqueous extracts, prepared by incubating 0.1 g of dried, ground leaf and root material in 15 mL of water for 1 h at 95 °C, and followed by filtration through a 0.80 μm filter. Chloride ion content in DW was quantified using ion meter equipped with chloride ion selective electrode (9617BNWP Chloride Combination Electrode, Thermo Fisher Scientific).

Total soluble carbohydrates quantification

Total soluble carbohydrates (TSC) were measured according to Dubois et al. (1995). Dried material (DW) was ground and mixed with 80% methanol on a rocker shaker for 24–48 h. Sulfuric acid and 5% phenol were added and mixed before absorbance readings were taken at 490 nm. TSC contents were expressed as 'mg equivalent of glucose' per gram of DW.

Chlorophyll and carotenoid content

Five mg of lyophilized and homogenized sample were extracted in 1.5 mL of 95% ethanol for 15 min and centrifuged at 2000 g (Universal 32R, Hettich, Germany) for 10 min. The clear supernatant (100 μL) was transferred to a 96-well microplate and absorbance was measured at 470, 648 and 664 nm spectrophotometrically with a microplate reader (Synergy II; Bio-Tek, USA). The concentrations of total chlorophyll a + b (Chl) and carotenoids (Car) were calculated according to the equations given by Lichtenthaler and Buschmann (2001), taking into account that the path length of microwells was filled to a quarter of their depth. Concentrations reported are averages of three biological replications, each consisting of two analytical replications, and the values were later converted to $mg\cdot g^{-1}$ DW.

Lipid peroxidation

Malondialdehyde (MDA) content was determined in 80% (v/v) methanol extract of 100 mg of fresh plant material (FW). MDA content was determined by the thiobarbituric acid-reactive-substances (TBARS) assay, modified to correct for the presence of interfering compounds, as described by Hodges et al. (1999).

Activity of antioxidant enzymes

The activity of enzymes was determined in the fresh leaves (FW) with the use of a Double Beam spectrophotometer U-2900 (Hitachi). Catalase (CAT) activity was measured according to the method described by Aebi (1984) and peroxidase (POD) activity by Lück (1962). 100 mg of fresh plant material (FW) was homogenized in 5 mL ice-cold phosphate buffers with pH 7.0 for CAT and pH 6.2 for POD. The samples were centrifuged at 4 °C for 15 min at 4800 g. For CAT activity analysis, 0.2 mL of the extract and 1.8 mL of the buffer were mixed with 1 mL of H₂O₂ solution in phosphate buffer. The absorbance of H₂O₂ decomposed by CAT was measured at 240 nm for 4 min at 1-min intervals. The unit of CAT activity was the amount of the enzyme that decomposed 1 µmol H₂O₂ in 1 min. For analysis of POD activity, p-phenyldiamine was used as the enzyme substrate, which is oxidized by POD to phenazine. One mL of the extract, 1 mL of phosphate buffer, 0.1 mL of 1% solution of p-phenyldiamine were mixed with 0.1 mL of 0.1% H₂O₂. The absorbance of colored reaction product was measured at 485 nm. One unit of POD activity corresponds to an absorbance increase of 0.1.

Non-enzymatic antioxidants

Total flavonoids (FV) were measured in methanol extracts (0.1 g of dried material in 3 mL of 80% methanol) and determined following the method described by Zhishen et al. (1999); the absorbance was measured at 510 nm, and the amount of flavonoids was expressed in equivalents of catechin (mg C eq·g⁻¹ DW), used as standard.

Total phenolic compounds (TPC) were determined according to the modified method of Singleton et al. (1999) using lyophilized plant material. A clear supernatant (50 µL) was diluted with 0.5 mL of deionized water and 0.2 mL of Folin-Ciocalteu reagent, and after

10 min 0.7 mL saturated Na₂CO₃ was added. The samples were incubated for 2 h, mixed and transferred to 96-well plates. Absorbance was measured at 765 nm on a micro-plate reader (Synergy 2, Bio-Tek Winooski, VT, USA). The level of total phenolics was expressed in equivalents of chlorogenic acid (CA) (mg CA eq·g⁻¹ DW) used as standard.

Metabolite profiling (determination of phytohormones, carbohydrates and stress-related amino acids)

Analyzes of soluble carbohydrates and endogenous phytohormones were conducted using HPLC (LC-MS) and UHPLC according to the same procedures as described earlier (Wiszniewska et al. 2019). The content of those metabolites in DW was measured in lyophilized leaf samples of the stressed plants and the corresponding controls. Details on equipment characteristics, compound extraction, separation and used standards are given in Supplementary Material 3. The measurements for each treatment were taken in 6 replicates. The carbohydrate profile included the following compounds: sugar alcohols: inositol, glycerol, arabitol, mannitol; oligosaccharides (including fructans): kestose, kestopentatose, nystose, raffinose, stachiose, maltotriose; mono- and disaccharides: glucose, fructose, sucrose, maltose; uronic acids: glucuronic acid, galacturonic acid. In the phytohormonal profiling the following PGRs were monitored: abscisic acid (ABA) (active: (±)-trans,trans-ABA, (±)-cis,trans-ABA, inactive: (±)-cis,trans-ABA glucosyl ester); 1-aminocyclopropane-1-carboxylic acid (ACC), auxins (active: indole-3-acetic acid, indole-3-butyric acid, 4-chloroindole-3-acetic acid, 5-chloroindole-3-acetic acid; conjugated: indole-3-acetyl-L-aspartic acid, indole-3-acetyl-L-glutamic acid, indole-3-carboxylic acid, indole-3-acetic acid methyl ester; oxidized: 2-oxo-3-indolineacetic acid); cytokinins (active: N6-isopentenyladenine, trans-zeatin, dihydrozeatin, cis-zeatin, meta-topolin, kinetin; trans-zeatin riboside, dihydrozeatin riboside, cis-zeatin riboside, kinetin riboside, m-topolin riboside, N6-isopentenyladenosine; deactivated: trans-zeatin-7-glucoside, trans-zeatin-O-glucoside); gibberellins (active: gibberellic acid, gibberellin A1, gibberellin A4; intermediate: gibberellins A5, A6, A7, A9; deactivated: gibberellin A8), jasmonic acid forms (active: (±)-jasmonic acid, (±)-jasmonic acid methyl ester; precursor: 12-oxo-phytodienoic acid) and salicylic and benzoic acids. Quantitations were based on calibration curves obtained

with pure standards of all compounds. Glycine betaine (GlyB), stachydrine (proline betaine, ProB) and free proline were estimated in the same samples simultaneously with ACC, and D-ACC was used as internal standard.

The detected carbohydrates and phytohormones were classified into groups and presented accordingly as described before (Wiszniewska et al. 2019).

Statistical analysis

The experiment was repeated three times. Ten pots with one plant per experimental treatment constituted a replicate. Data were statistically analyzed using STATISTICA 13.0 software (StatSoft, Tulsa, OK, USA): one way ANOVA to assess differences between the species-specific responses to progressive salt concentrations, and to compare reaction of each species to particular salinity level. Correlation matrix was applied to determine the level of correlation between the changes in PGR content and changes in individual biochemical parameters.

Results

Profile of plant growth regulators in plants subjected to salinity

Auxins

Under salinity total level of auxins (Aux) increased substantially in *A. alpinus*. However, only 3–8% of this pool accounted for active compounds, depending on the salinity level (Table 1, Suppl. Table 2). Increments in the pool of inactive Aux (both conjugated and oxidized) begun yet under low salinity. Contrary to that, *A. tripolium* was capable of maintaining constant content of active and inactive (oxidized and conjugated) Aux under low and moderate salinity in comparison to control conditions. Aux inactivation level increased only under high salinity. In both species, the proportions between the pool of active and inactive Aux were similar (Table 1).

Cytokinins

Concentrations of cytokinins (Cyt) were not affected by any of the applied salinity level in either species, as

compared to untreated plants (Table 1). The prevailing compound was intermediate Cyt in a form of ribosides (four times more abundant than inactive Cyt-glucosides and twice as abundant as the active forms). The concentrations amounted to 0.8–0.9 nmol g⁻¹ DW in *A. tripolium* and 0.9–1.0 nmol g⁻¹ DW in *A. alpinus* ($P > 0.05$).

Gibberellins

In both studied species, accumulation of active and inactive gibberellins (GA) in response to salinity was not influenced by increasing external NaCl concentrations (Table 1). The pool of active GA differed among the species under moderate salinity, whereas the content of inactive GA was higher in halophyte under high salinity conditions. Salinity affected accumulation of GA intermediates, the contents of which increased in *A. tripolium* under moderate and high salinity (Table 1). In *A. alpinus*, the pool of GA intermediates increased under low and moderate salinity ($P > 0.05$) while in plants exposed to high salt stress it was the same as in the control. Regardless salinity level, the content of GA intermediates was higher in *A. alpinus* than in *A. tripolium* (Table 1).

Stress hormones: abscisic acid, jasmonates and ethylene precursor

Control *A. tripolium* plants had high initial ABA concentration, which declined under low and moderate salinity and increased again under high salinity (Table 2, Suppl. Table 1). An opposite trend was observed in *A. alpinus*, where initial concentration of active ABA forms was low (11 nmol g⁻¹ DW), and increased under low and moderate salinity (Table 2). Under high salinity ABA content was the same as in the control. In this species, there was a decrement in inactive ABA-glucoside content that was not observed in *A. tripolium*.

Initial concentration of active JA did not differ between *A. tripolium* and *A. alpinus*, and species reactions to salinity were similar (Table 2). The content of active JA declined, while jasmonic acid precursor – oxophytodienoic acid (OPDA) increased in salt-treated plants, regardless of salinity intensity.

In control conditions the content of ethylene precursor (1-aminocyclopropane-1-carboxylic acid, ACC) was comparable in *A. tripolium* and *A. alpinus*. It

Table 1 The content of endogenous growth-promoting phytohormones in *A. tripolium* and *A. alpinus* subjected to salinity

Salinity level ^a	Endogenous phytohormones (nmol·g ⁻¹ DW)								
	Auxins			Cytokinins			Gibberellins		
	Actv ^b	Deact ^c Oxo ^d	Conj ^e	Actv	Inter ^f	Deact	Actv	Inter	Deact
<i>A. tripolium</i>									
none	1.4±0.1aA	1.3±0.2 bA	12.5±4.4aA	0.4±0.0aB	0.9±0.1aA	0.04±0.0aA	2.0±0.9aA	7.3±0.7aA	1.6±0.1aA
low	1.1±0.2aA	1.2±0.1bA	10.8±2.2aA	0.4±0.0aA	0.8±0.0aA	0.1±0.0aA	3.2±1.1aA	11.9±3.1aA	2.6±0.3aA
moderate	1.7±0.1aA	0.3±0.0aA	11.9±2.5aA	0.3±0.0aA	0.9±0.0aA	0.1±0.0aA	0.7±0.2aA	18.2±1.1bA	2.3±2.0aA
high	3.2±1.8bA	2.7±1.9cA	59.1±0.6bA	0.5±0.3aB	0.9±0.3aA	0.04±0.0aA	1.9±1.6aA	23.1±5.4cA	3.7±0.9aB
<i>A. alpinus</i>									
none	3.0±0.1aB	0.7±0.1aA	5.5±1.8aA	0.2±0.2aA	1.0±0.0aA	0.1±0.01aB	2.1±1.0aA	16.95±2.1aB	1.7±1.3aA
low	5.1±0.3 bB	2.2±0.2bA	52.7±2.3bB	0.3±0.0aA	1.0±0.1aB	0.1±0aB	2.1±0.7aA	29.85±0.2bB	2.9±1.5aA
moderate	5.2±0.3bB	3.3±1.9cB	61.9±11.2bB	0.3±0.0aA	1.0±0.0aA	0.1±0.02aA	3.4±1.5aB	23.39±8.5bB	2.5±1.2aA
high	3.0±0.4aA	4.4±0.5 dB	79.4±6.03cB	0.3±0.0aA	0.9±0.1aA	0.1±0aA	1.4±0.9aA	11.74±4.0aB	1.9±0.7aA

Values are means ± SE (n = 6). For each species, different lower case letters indicate significant differences among treatments and different capital letters indicate significant differences among species undergoing the same treatment, according to Tukey's test ($P \leq 0.05$)

^a salinity levels were assigned as none (no NaCl applied), low (200 mM NaCl), moderate (400 mM NaCl) and high (600 mM NaCl)

^b Active compounds, ^c Deactivated compounds, ^d Oxidized compounds, ^e Conjugates, ^f Intermediate compounds

significantly decreased in both species under salinity. The decline was more pronounced in *A. tripolium* (ACC concentration 2–3 times lower in stressed than in the control plants), than in *A. alpinus* (1.5-fold decrease) (Table 2).

Benzoic and salicylic acid

Under low and moderate salinity, the content of benzoic acid (BA) was the same as in control conditions, with total BA content higher in *A. tripolium* than in

Table 2 The content of endogenous stress-related phytohormones in *A. tripolium* and *A. alpinus* subjected to salinity

Salinity level ^a	Endogenous phytohormones (nmol·g ⁻¹ DW)				
	Absciscic acid		Jasmonates		Ethylene precursor
	Actv ^b	Deact ^c	Actv	Precursor	ACC
<i>A. tripolium</i>					
none	53.3±6.4bB	8.7±1.9bA	11.2±1.0bA	1.1±0.0aA	228.3±54.1bA
low	27.3±2.9aA	6.4±1.0bA	4.8±0.1aA	1.6±0.5bA	64.4±24.2aA
moderate	30.9±1.7aA	7.4±0.2bA	1.3±0.0aA	1.5±0.2bA	61.0±45.8aA
high	48.6±13.3bB	4.8±2.5aA	0.5±0.2aA	2.4±0.8bA	97.7±57.0aA
<i>A. alpinus</i>					
none	11.0±0.4aA	16.4±1.2bB	9.6±3.1bA	0.8±0.3aA	313.8±59.7bA
low	26.2±0.7bA	14.6±0.1bB	1.1±0.3aA	1.6±0.4bA	170.5±35.8aB
moderate	20.5±5.6bA	11.2±5.4bA	1.0±0.3aA	1.7±0.1bA	193.5±55.5aB
high	14.4±1.4aA	6.3±0.8aA	0.9±0.1aA	1.7±0.1bA	208.7±46.2abB

Values are means ± SE (n = 6). For each species, different lower case letters indicate significant differences among treatments and different capital letters indicate significant differences among species undergoing the same treatment, according to Tukey's test ($P \leq 0.05$)

^a salinity levels were assigned as none (no NaCl applied), low (200 mM NaCl), moderate (400 mM NaCl) and high (600 mM NaCl)

^b Active compounds, ^c Deactivated compounds,

A. alpinus (Fig. 1a). In high salinity conditions it decreased in *A. tripolium* and increased in *A. alpinus*, so final BA concentration in *A. alpinus* was higher than in *A. tripolium*. Salicylic acid (SA) content rose in *A. alpinus* at all salinity levels (regardless of its intensity), while in *A. tripolium* SA accumulation significantly dropped at low and moderate salinity levels (Fig. 1b). In salt-treated *A. alpinus* the content of SA was higher than in respective *A. tripolium* plants. Considering the entire pool of growth-regulating compounds, it is noteworthy that the changes in BA and SA contents, together with distinct pattern of active ABA accumulation, were the parameters that clearly diversified responses to salinity between the studied species.

Growth parameters and chlorophyll content

The stem length (SL), expressed as a percentage of respective untreated controls, decreased in both studied

species exposed to salinity. Average SL reduced about 35 to 60% and 43 to 73% of the control in *A. tripolium* and *A. alpinus*, respectively (Table 3a).

Reduction of water content (WC) in the leaves was more pronounced in salt-sensitive *A. alpinus* than in *A. tripolium*. Under low, moderate and high salinity water content loss in the leaves of *A. alpinus* was 6%, 23% and 27%, respectively, in comparison with control plants. In *A. tripolium*, WC% decreased significantly only at high salinity (Table 3b).

Growth Tolerance Index (GTI) (calculated on the basis of DW) for shoots exposed to low, moderate and high salinity amounted to 99, 82 and 83%, respectively, in *A. tripolium*, and to 59, 67 and 70% in *A. alpinus*. In *A. alpinus* differences in GTI between salinity levels were statistically insignificant (Table 3c).

Chlorophyll content (Chl) was here considered a parameter that reflected plant photosynthetic capacity and species ability to sustain growth under stress.

Fig. 1 Organic acids content in two Asteraceae species subjected to salinity. Benzoic acid (BA) (a), salicylic acid (SA) (b) content in leaves of two Asteraceae species, after 4 weeks of salt treatments of exposure to low, moderate and high salinity. Values are means $\pm$ SE ($n = 6$). For each species, different lower case letters indicate significant differences among treatments and different capital letters indicate significant differences among species undergoing the same treatment, according to ANOVA and post hoc Tukey's test ($P \leq 0.05$)

Table 3 Growth parameters and chlorophyll content in *A. tripolium* and *A. alpinus* subjected to salinity

Parameter	Salinity treatments ^a	Species	
		<i>A. tripolium</i>	<i>A. alpinus</i>
(a) Stem length (% of control)	none	100±3 cA	100±3.61 cA
	low	65.85±4.58 bA	57.14±2.29 bA
	moderate	52.85±3.51 bA	44.44±1.53 bA
	high	39.02±3.61 aB	27.14±0.44 aA
(b) WC% in leaves	none	84.98±0.36 bA	70.89±6.68 cA
	low	86.95±1.11 bB	65.22±13.43 bA
	moderate	80.21±3.49 bB	47.67±5.65 aA
	high	63.24±9.74 aB	43.65±5.27 aA
(c) GTI shoots	none	100	100
	low	99.32 bA	59.53 aB
	moderate	82.46 aA	66.98 aB
	high	82.93 aA	69.78 aB
(d) Chl _{a+b} (mg·g ⁻¹ DW)	none	8.95±0.03 dB	6.54±0cA
	low	7.4±0.15cB	1.57±0.01bA
	moderate	4.38±0.31bB	0.25±0.11aA
	high	1.24±0.03aB	0.22±0.05aA

Values are means ±SE ($n = 3$). For each species, different lower case letters indicate significant differences among treatments and different capital letters indicate significant differences among species undergoing the same treatment, according to one-way ANOVA and post hoc Tukey's test ($P \leq 0.05$)

^a salinity levels were assigned as none (no NaCl applied), low (200 mM NaCl), moderate (400 mM NaCl) and high (600 mM NaCl)

Salinity reduced the leaf content of chlorophylls (a + b) in both *Aster* species, but more pronounced decrease was detected in salt-treated *A. alpinus*. Under low salinity, the decline reached 18 and 76%, while under high salinity 86 and 90% in *A. tripolium* and *A. alpinus*, respectively (Table 3d).

Correlations between growth parameters and changes in PGR content

Correlations between data on growth parameters and on phytohormones were identified using a correlation matrix. Several differences in correlating parameters were observed between the studied species (Fig. 2). On the Fig. 2 PGRs that were predominantly correlated with studied parameters in halophyte *A. tripolium* were colored blue, while those that were mainly correlated with the parameters determined in glycophyte *A. alpinus* were colored red.

In halophyte stem length (SL), water content percentage (WC%) and total chlorophylls (Chl) positively correlated with BA content, and SL and Chl also with JA

content. Majority of growth parameters negatively correlated with total GA pool, and WC% and Chl also with Cyt and Aux. Only GTI positively correlated with the changes in SA content.

In turn, in glycophyte all growth parameters (SL, WC%, GTI, Chl) negatively correlated with the contents of salicylic acid (SA) and ACC (ethylene precursor).

Ion accumulation

Sodium and chloride levels increased in the roots and leaves of both species, in parallel with increasing external salinity, in comparison with the control conditions. *A. tripolium* accumulated more Na⁺ in its organs than *A. alpinus* (Fig. 3a). In the roots treated with low, moderate and high salinity *A. alpinus* accumulated 16, 26 and 40 g·kg⁻¹ DW, respectively, as compared with 22, 51 and 74 g·kg⁻¹ DW determined for *A. tripolium*. A similar trend was observed in the leaves. In *A. tripolium* roots, Na⁺ concentrations in the control and under low salinity were similar ($P > 0.05$). Interestingly, the initial Na⁺ content in *A. alpinus* was 4.5 times and 7 times

		Halophyte <i>Aster tripolium</i>		Glycophyte <i>Aster alpinus</i>	
		(+)	(-)	(+)	(-)
Growth performance	SL	BA, JA	GA		SA, ACC
	WC%	BA	GA, Aux, Cyt		SA, ACC
	GTI	SA			SA, ACC
	Chl	BA, JA	GA, Aux, Cyt		SA, ACC
Osmolytes	Pro	GA	BA, JA		
	GlyB	GA	BA, JA		BA
	ProB	GA, Aux, Cyt	BA	ABA, ACC	
	TSC	BA, Cyt, GA	Aux	ABA	Cyt, GA
	SuAlc	GA	JA, BA	GA, BA, Cyt, ABA	
	Oligo	JA	GA		
	Fruct	JA	GA		
	Mono & Di	GA	JA, BA	SA, ACC	
	UA		SA		Cyt, GA, ABA
Ions	Na Leaf	Cyt, Aux, GA	JA, BA		
	Na Root	Cyt, Aux, GA	JA, BA		
	Cl Leaf	Cyt, GA	JA, BA		Cyt, GA, ABA, SA
	Cl Root	Cyt, Aux, GA	JA, BA		Cyt, GA, ABA, SA
	K Leaf	JA, BA	Cyt, GA		ACC
	K Root	JA, BA	Cyt, Aux, GA		ACC
Antioxidant machinery	MDA	Cyt, Aux, GA	BA		SA, ACC
	POD		Cyt, Aux, GA	Cyt, GA, ABA, SA	
	CAT		Cyt, GA		
	TPC		JA, BA		ACC
	FV	JA, BA	SA	ACC, SA	
	Car		JA, BA		ACC

(+) positive correlation, (-) negative correlation, abbreviations defined in the main text

Fig. 2 Significant correlations (>0.75 , <-0.75) between the changes in endogenous PGRs content and determined biometrical and biochemical parameters in halophyte *A. tripolium* (blue) and glycophyte *A. alpinus* (red) under progressive salinity

lower in the roots and the leaves, respectively, in comparison with control *A. tripolium* plants (Fig. 3a).

Chloride content at respective salinity levels was always lower in *A. alpinus* than in *A. tripolium*. Also, initial Cl^- concentrations were lower in *A. alpinus* roots (~2- fold) and leaves (~3-fold), as compared with *A. tripolium*. Cl^- content in *A. tripolium* roots remained at the control level at low salinity. In turn, in the roots of *A. alpinus* similar accumulation of Cl^- was noted at moderate and high salinity (Fig. 3b).

Potassium content in *A. tripolium* roots was stable under low and moderate salinity, and declined significantly at high salinity. In *A. alpinus* roots K^+ content increased at low salinity, while at moderate and high stress it was at the same level as in control plants (Fig. 3c). Similarly to Na^+ , the initial K^+ content in the organs of control plants was significantly higher in *A. tripolium* than in *A. alpinus* (37.63 vs. 21.2 $\text{g}\cdot\text{kg}^{-1}$ DW in the roots and 72.44 vs. 45.3 $\text{g}\cdot\text{kg}^{-1}$ DW in the leaves, respectively). The studied species differed also in a pattern of K^+ accumulation in the leaves. In *A. tripolium* K^+ content decreased in parallel with increasing salinity, while in *A. alpinus* it remained unaffected (Fig. 3c). Due to significant and progressive reduction of K^+ content in

A. tripolium leaves, its concentration at high salinity reached about 50% of the control (Fig. 3c). However, it was still higher than in any of *A. alpinus* leaves.

The K^+/Na^+ ratio, which is considered a parameter relevant for maintaining ionic homeostasis, generally decreased in the leaves and the roots of both species under salinity conditions, with an exception of *A. tripolium* roots exposed to low salinity (Fig. 3d). Initially the ratio was higher in *A. alpinus* than in *A. tripolium*, and reductions caused by salinity were also more pronounced in *A. alpinus*.

Correlations between ion content and changes in PGR content

In halophyte sodium and chloride contents in plant organs positively correlated with total Cyt, Aux and GA pools, and negatively with JA and BA content. A reverse pattern of correlations was found in the case of potassium ions – a positive correlation with JA and BA, and negative with Cyt, Aux and GA concentrations (Fig. 2).

In glycophyte sodium concentrations in plant organs did not correlate with the content of any PGR, whereas

Fig. 3 Ion accumulation in two Asteraceae species subjected to salinity. Sodium (Na⁺) (a), chloride (Cl⁻) (b), potassium (K⁺) (c) concentrations and K⁺/Na⁺ ratio (d) in leaves and roots of two Asteraceae species, after 4 weeks of exposure to low, moderate and high salinity. Values are means ± SE (n = 6). For each species,

different lower case letters indicate significant differences among treatments and different capital letters indicate significant differences among species undergoing the same treatment, according to ANOVA and post hoc Tukey's test (P ≤ 0.05)

chloride accumulation negatively correlated with the contents of Cyt, GA, ABA and SA. Potassium content negatively correlated with the level of ACC (Fig. 2).

Accumulation of osmolytes: osmoprotectant amino acids

Proline (Pro) content increased under salinity in both species, however, a 2-fold higher increment was recorded in *A. alpinus* than in *A. tripolium* under the highest salinity (2056 μg·g⁻¹ DW and 1083 μg·g⁻¹ DW respectively). The initial Pro content was similar in both species (Table 4).

Regarding glycine betaine (GlyB) accumulation, no clear trend was found in any of the studied species. In *A. tripolium*, an increase in GlyB was observed at low salinity, no changes at moderate

one and a slight decrease at high stress level (Table 4). In *A. alpinus*, a decline in GlyB content was recorded under all applied salinity levels, and it was more pronounced under low and moderate salinity than under the high one. The initial GlyB level was over 3-fold higher in *A. alpinus* than in *A. tripolium* (Table 4).

Proline betaine (ProB) content was very low (not exceeding 2 μg·g⁻¹ DW) in *A. tripolium* and no significant changes were observed under salt exposure. Contrary to that, in *A. alpinus* a drastic increment of this amino acid content was noted (303, 321 and 1106 μg·g⁻¹ DW under low, moderate and high salinity, respectively) in comparison with 3 μg·g⁻¹ DW in the control (Table 4). ProB was the only amino acid whose content changed differently under salinity in the studied species.

Table 4 Proline (Pro), glycine betaine (GlyB), proline betaine (ProB) concentrations in two Asteraceae species subjected to salinity

Salinity level ^a	Osmoprotectant amino acids ($\mu\text{g}\cdot\text{g}^{-1}$ DW)		
	Pro	GlyB	ProB
<i>A. tripolium</i>			
none	41.7 $\pm$ 13.2 aA	1199.5 $\pm$ 206.8 bA	1.4 $\pm$ 0.0 aA
low	508.8 $\pm$ 116.1 bA	2192.4 $\pm$ 361.3 cB	1.2 $\pm$ 0.0 aA
moderate	1014.3 $\pm$ 111.6 cA	1169.0 $\pm$ 234.9 bA	1.4 $\pm$ 0.0 aA
high	1083.0 $\pm$ 289.9 cA	689.5 $\pm$ 126.7 aA	1.9 $\pm$ 1.6 aA
<i>A. alpinus</i>			
none	79.3 $\pm$ 32.56 aA	3590.8 $\pm$ 126.7 cB	3.2 $\pm$ 0.1 aA
low	1132.2 $\pm$ 57 bB	1541.9 $\pm$ 238.8 aA	303.2 $\pm$ 9.6bB
moderate	986.8 $\pm$ 151.30 bA	1187.8 $\pm$ 224.1 aA	321.6 $\pm$ 10.7 bB
high	2056.9 $\pm$ 59.75 cB	2833.3 $\pm$ 95.5 bB	1106.9 $\pm$ 12.9 bB

Values are means $\pm$ SE, (n = 6). For each species, different lower case letters indicate significant differences among treatments and different capital letters indicate significant differences among species undergoing the same treatment, according to ANOVA and post hoc Tukey's test ($P \leq 0.05$)

^a salinity levels were assigned as none (no NaCl applied), low (200 mM NaCl), moderate (400 mM NaCl) and high (600 mM NaCl)

Correlations between the content of osmoprotectant amino acids and PGRs

In halophyte the concentrations of all determined amino acids (Pro, GlyB, ProB) negatively correlated with BA content, and Pro and GlyB also with JA. A positive correlation occurred between the contents of all amino acids and GA. Additionally, ProB content positively correlated with Aux and Cyt (Fig. 2).

In glycophyte GlyB content negatively correlated with changes in BA level, while ProB correlated positively with ABA and ACC content. Changes in Pro levels did not correlate with any changes in the profile of endogenous PGRs (Fig. 2).

Accumulation of osmolytes: carbohydrates

In investigated species total soluble carbohydrate (TSC) content was affected by salinity in distinct manner. In *A. tripolium*, TSC decreased under high salinity, and did not change under low and moderate salinity. In *A. alpinus* TSC increased in salinity conditions in comparison with the control, regardless of stress intensity (Fig. 4a). TSC content differed between *A. tripolium* and *A. alpinus* only at high salinity, where it was higher in the latter species (Fig. 4a).

Oligosaccharides

Total pool of oligosaccharides, including fructans, decreased under salinity in both studied species. Interestingly, the initial concentrations of these compounds were considerably higher in *A. alpinus* than in *A. tripolium*. Oligosaccharides amounted to 12.33 and 13.23 $\mu\text{g}\cdot\text{mg}^{-1}$ DW in *A. tripolium* and *A. alpinus*, respectively, while the levels of fructans reached 4.17 and 6.39 $\mu\text{g}\cdot\text{mg}^{-1}$ DW (data not shown).

Sugar alcohols

In *A. alpinus* salinity did not change the content of sugar alcohols, which was considerably low (about 3 $\mu\text{g}\cdot\text{mg}^{-1}$ DW) (Fig. 4c). Contrary to that, *A. tripolium* responded to salinity with massive accumulation of sugar alcohols. The content of these compounds increased to 18.5–26.8 $\mu\text{g}\cdot\text{mg}^{-1}$ DW, in comparison with 5.2 $\mu\text{g}\cdot\text{mg}^{-1}$ DW in control plants ($P < 0.05$). Polyol content was the carbohydrate profile parameter that diversified responses to salinity in the studied species.

Mono- and disaccharides

A clear salinity-dependent increase in glucose and fructose accumulation was only detected in *A. alpinus*, whereas in *A. tripolium* changes in the concentrations

Fig. 4 Carbohydrate profile in two Asteraceae species subjected to salinity. Content of total soluble carbohydrates (TSC) (a), oligosaccharides (b), sugar alcohols (c), mono- and disaccharides (d) and uronic acids (e) in two Asteraceae species, after 4 weeks of exposure to low, moderate and high salinity. Values are means

$\pm$ SE ($n = 6$). For each species, different lower case letters indicate significant differences among treatments and different capital letters indicate significant differences among species undergoing the same treatment, according to ANOVA and post hoc Tukey's test ($P \leq 0.05$)

of both sugars were statistically insignificant (data not shown). Regarding sucrose, a considerable increase was detected in *A. tripolium* under moderate and high salinity, whereas in *A. alpinus* sucrose content drastically increased under low salinity and decreased under moderate and high one (data not shown). Maltose content was mostly affected by salinity treatment in *A. alpinus* (its levels dropped from $13.23 \mu\text{g}\cdot\text{mg}^{-1}$ DW in control plants to $4.84\text{--}6.07 \mu\text{g}\cdot\text{mg}^{-1}$ DW, $P < 0.05$) (data not shown). Regarding total content of mono- and

disaccharides, it increased in both species under salinity, with only exception of *A. tripolium* subjected to its low level (no change in comparison with untreated plants). Accumulation of these carbohydrates was more intense in *A. alpinus* than in *A. tripolium* (Fig. 4d).

Uronic acids

Concentrations of uronic acids (UA) in the control plants were relatively low and comparable in both

Fig. 5 Lipid peroxidation, a content of non-enzymatic antioxidants and antioxidant enzyme activity in two Asteraceae species subjected to salinity. Leaf malondialdehyde (MDA) (a), total flavonoids (FV) (b), total phenolic compounds (TPC) (c), carotenoids (Car) (d) contents, catalase activity (e) and peroxidase activity (f) in two Asteraceae species, after 4 weeks of exposure

to low, moderate and high salinity. Values are means $\pm$ SE ($n = 6$). For each species, different lower case letters indicate significant differences among treatments and different capital letters indicate significant differences among species undergoing the same treatment, according to ANOVA and post hoc Tukey's test ($P \leq 0.05$)

studied species (Fig. 4e). Accumulation of these compounds accelerated in response to salinity. Particularly in *A. tripolium*, total UA level increased from $4 \mu\text{g}\cdot\text{mg}^{-1}$

DW in control plants to 135.5 and $125.6 \mu\text{g}\cdot\text{mg}^{-1}$ DW under moderate and high salinity, respectively (Fig. 4e). In *A. alpinus*, increments were not as high as in

A. tripolium and reached 41.8–55.2 $\mu\text{g}\cdot\text{mg}^{-1}$ DW (Fig. 3e).

Correlations between the content of carbohydrates and PGRs

In halophyte TSC positively correlated with Cyt, GA and BA pools and negatively with Aux. Accumulation of oligosaccharides (Oligo) (including fructans (Fruc)) correlated positively with JA and negatively with total GA pool. Inverse correlations occurred for the content of sugar alcohols and mono- and disaccharides that correlated positively with GA and negatively with JA levels. The content of sugar alcohols (SuAlc) also negatively correlated with changes in BA content. Uronic acids (UA), massively accumulated in halophyte under salinity, negatively correlated with SA content (Fig. 2).

In glycophyte TSC negatively correlated with the contents of Cyt and GA, and positively with ABA. Positive correlation was also found between accumulation of sugar alcohols and changes in GA, Cyt, ABA and BA contents, as well as between the contents of mono- and disaccharides and SA and ACC. A negative correlation occurred between the contents of uronic acids and the pool of Cyt, GA and ABA (Fig. 2).

Oxidative stress level and antioxidant activity

In control conditions, malondialdehyde (MDA) concentration, reflecting oxidative stress level, was higher in *A. tripolium* than in *A. alpinus*. Under salinity, regardless of its level, MDA content increased significantly in *A. alpinus*, while in *A. tripolium* lipid peroxidation enhanced only under high salinity (Fig. 5a).

As for non-enzymatic antioxidants, both species increased their accumulation of total flavonoids (FV) under moderate and high salinity, and in case of *A. alpinus* at low salinity as well. The increase was more pronounced in *A. alpinus* and occurred at all salinity levels. In *A. tripolium*, significant increments in FV content occurred under moderate and high salinity (Fig. 5b). An inverse trend was observed for total phenolics (Fig. 5c) and carotenoids (Fig. 5d). In control conditions, *A. tripolium* had higher content of these compounds than *A. alpinus*. Salinity reduced TPC and Car accumulation in both species, with lower contents determined in *A. alpinus* than in *A. tripolium* under respective treatments (Fig. 5 cd).

The studied species exhibited different patterns of two antioxidant enzymes activity in response to salinity. In *A. alpinus*, the enzymatic activity increased under salinity conditions, irrespectively of NaCl concentrations. CAT activity increased 3-fold and POD 1.5- fold in comparison with control plants (Fig. 5e, f). In *A. tripolium*, the activity of CAT did not change under salinity, whereas POD activity declined under moderate and high salinity by 36 and 62% respectively (Fig. 5 e, f).

Correlations between the elements of antioxidant machinery and changes of PGRs content

In halophyte the content of MDA correlated positively with total concentrations of Cyt, Aux and GA, and negatively with BA levels. Significant correlations between PGRs and elements of antioxidant machinery were also found. Both POD and CAT activity negatively correlated with total pool of Cyt and GA, and POD also correlated with Aux. The contents of non-enzymatic antioxidants, TPC and Car, correlated negatively with JA and BA levels, whereas FV content correlated positively with these two PGRs. Flavonoids also negatively correlated with SA content.

In glycophyte negative correlation was found between MDA content and the concentrations of SA and ACC. Considering the antioxidant system, POD activity correlated positively with total pool of Cyt, GA, ABA, and SA. In the case of CAT activity, no significant correlations with PGR content were detected. Concentrations of non-enzymatic antioxidants correlated with ACC accumulation: TPC and Car – negatively, and FV – positively. Additionally, changes in FV concentrations correlated positively with SA concentrations.

Discussion

Halophyte adaptation to salinity requires the interplay between three known defense mechanisms: maintaining of ion homeostasis, osmotic adjustment by synthesis of osmolytes, and effective neutralization of excessive reactive oxygen species (ROS) by antioxidant system (Soundararajan et al. 2019). The same mechanisms, although usually less pronounced, are activated in salt-sensitive glycophytes subjected to salt excess (Bartels and Dinakar 2013; Ellouzi et al. 2014; Gharbi et al. 2017; Shabala 2013). Differences between the

responses of halophytes and glycophytes, resulting in salinity tolerance or its lack, can be conditioned by subtle variations in regulatory and signaling pathways, yet they are poorly understood. Recently, metabolomic profiling, including profiling of endogenous phytohormones, brought about new information on these aspects in salt-tolerant species (Benjamin et al. 2019; Kumari and Parida 2018; Wiszniewska et al. 2019). In this study, a comparative analysis of defense mechanisms under low, moderate and high salinity was aimed at correlating the changes in the level of several parameters, depicting defense mechanisms, with the changes occurring in the pools of major PGRs. This approach revealed that in halophytic and glycophytic Asteraceae species distinct regulatory compounds are responsible for developing defense response under salt exposure.

Ion uptake and maintaining ionic homeostasis

Our study revealed that *A. tripolium* had higher initial content of Na^+ , K^+ and Cl^- ions than *A. alpinus*. In this respect, a classification of the studied species into halophyte and glycophyte was substantiated (Cheeseman 2015). Under salinity, both species accumulated Na^+ and Cl^- and translocated them to the shoots, but in the halophyte these processes were far more intense than in the glycophyte. Contrary to this study, other halophytes were found to restrict Na^+ and Cl^- uptake in comparison with non-tolerant species (Ellouzi et al. 2014; Prerostova et al. 2017). Our results could be attributed to the species-specific requirement for maintaining high Na^+ in order to activate water-saving mechanisms (Kersters et al. 2002). High capacity to accumulate Na^+ and Cl^- without serious growth inhibition can be a promising feature for future exploitation of *A. tripolium* in phytodesalination experiments on low and moderately saline soils.

The investigated species had distinct patterns of K^+ concentration. In the halophyte, its initial content was over twice as high as in the glycophyte, and it declined under moderate and high salinity. Generally, halophytes are reported to accelerate K^+ uptake when exposed to saline soil (Gharbi et al. 2017). However, in *A. tripolium*, the initial K^+ content seems to be high enough to assure normal functioning, even if K^+ concentration drops due to excessive Na^+ uptake and competitiveness for binding sites in ion transporters (Wang

et al. 2013). It is also possible that in salt-exposed *A. tripolium* Na^+ may be a more crucial ion than K^+ , as reported for halophyte *S. portulacastrum* (Wang et al. 2012). Since elevated Na^+ amounts are toxic for both glycophytes and halophytes, the latter species usually have more efficient of Na^+ detoxification mechanism based on sequestering the ions in the vacuole (Ali and Yun 2017). However, even under high salinity, where the most pronounced decrease occurred, K^+ content still exceeded the content in the glycophyte. The response of *A. alpinus* to salinity was the same as in other non-salt tolerant species. K^+ content did not change, which contributed to deregulation of ion homeostasis caused by enhanced Na^+ uptake (Ellouzi et al. 2014).

Osmotic adjustment and osmoprotection

Osmotic adjustment via accumulation of osmolytes is a common response to salinity activated in both tolerant and sensitive species (Gong et al. 2005). We found that in salt-tolerant *A. tripolium* the main changes in the carbohydrate profile involved intense accumulation of uronic acids, mannitol, and, to a lower extent, sucrose. Uronic acids provide efficient water storage and are capable of neutralizing Na^+ excess (Jaiswar and Kazi 2016). Functions of mannitol in salt-tolerant organisms, particularly alga, involve osmoprotection and radical scavenging (Patel and Williamson 2016). Although its accumulation is rarely observed in halophytes (Slama et al. 2015), we previously reported that in *A. tripolium* mannitol was specifically accumulated under salt exposure, and was not up-synthesized in response to metallic stress (neither Cd nor Pb-induced) (Wiszniewska et al. 2019). In this respect, mannitol synthesis under salinity could be regarded as an adaptive mechanism of osmoprotection in the studied halophyte. It can be also assumed that carbohydrate accumulation pattern in salt-exposed *A. tripolium* is oriented towards protection of cell compartments and proteins from dehydration.

Carbohydrate profiling in glycophytic *A. alpinus* showed that salinity enhanced accumulation of total soluble carbohydrates, mono- and disaccharides, and uronic acids. Glucose accumulation played the major role under salinity, and the accumulation of sucrose was less intense (data not shown). This was inevitably directed towards osmotic adjustment, but also could be linked to energy requirements and regulatory pathways under stress (Dong and Beckles 2019; Hu et al. 2012). In this species, polyol content did not change under

salinity, suggesting the absence of similar osmoprotection mechanisms as observed in *A. tripolium*.

Both species experienced depletion of oligosaccharide content under salinity. The main fraction in this group were fructans. Non-stressed *A. alpinus* was particularly rich in kestose and nystose, the levels of which dropped dramatically under salinity. Storage function of these inuline-type oligosaccharides was replaced by their utilization as carbon source and potential ROS scavengers (Dong and Beckles 2019; Van den Ende 2013).

Osmotic adjustment under salinity involved also amino acid accumulation, mainly by enhanced synthesis of proline and betaines, and such a response was more pronounced in the glycophyte than in the halophyte. This involved particularly ProB synthesis, which was virtually absent in the halophyte unless high salinity was applied. Salinity tolerance in halophytes is usually attributed to their higher content of Pro and betaines, as well as to the ability of their rapid and intensive synthesis (Benjamin et al. 2019; Ellouzi et al. 2014; Pardo-Domènech et al. 2016). Our study showed that glycophytic *A. alpinus* had also considerable potential to counteract dehydration by intensification of amino acid accumulation. Proline is usually synthesized for rapid utilization, while betaines, particularly ProB, for prolonged storage (Slama et al. 2015). Both routes of amino acid utilization were activated in the glycophyte, while in the halophyte the first one prevailed. Although accumulation of betaines was pronounced in the glycophyte, it did not correspond to higher antioxidant capacity, as reported for other species (Rady et al. 2018; Youssef et al. 2018). Taking into account high initial content of these osmoprotectants in *A. alpinus*, further studies on the species abiotic stress resistance are substantiated.

Counteracting oxidative stress

We observed differences in the redox state and functioning of antioxidant system under salinity conditions between the studied species. The halophyte had initially higher content of MDA, what is usually attributed to higher level of oxidative damage (Anjum et al. 2015). However, control MDA concentrations (below $20 \text{ nmol} \cdot \text{g}^{-1} \text{ DW}$) were still considerably low, suggesting occurrence of typical cellular metabolism, as in salt-tolerant *Cakile maritima* and *Thellungiella salsuginea*

(Ellouzi et al. 2014). Hence, in *A. tripolium* oxidative stress was inefficiently counterbalanced only at high salinity conditions. This is corroborated by the fact that the halophyte had very high initial activity of CAT and POD, in comparison with the glycophyte, which did not change under low salinity and only partially (POD) at moderate salinity. Under high salinity, POD activity declined substantially, and simultaneously MDA content rapidly increased. Also the contents of non-enzymatic antioxidants, both total phenolics and carotenoids, were considerably higher in the halophyte than in the glycophyte subjected to respective salinity conditions. Although in both species TPC and Car concentrations progressively declined with increasing salinity, their levels were higher in the halophyte than in the glycophyte. It seems that boosted activity of antioxidant enzymes and enhanced accumulation of non-enzymatic antioxidants were involved in constitutive protective mechanisms under low and moderate salinity in *A. tripolium*. This is in agreement with previous studies reporting that metabolites responsible for stress defense mechanisms are usually more abundant in pre-stress conditions in salt-resistant than in salt-sensitive species (Gharbi et al. 2017; Gong et al. 2005; Prerostova et al. 2017). An interesting exception from this pattern observed in our study was accumulation of flavonoids. Their initial content was higher in the glycophytic *A. alpinus* in comparison with *A. tripolium*. Although in both species enhanced synthesis of FV occurred as a response to salinity treatments, their content in the glycophyte always exceeded that in the halophyte. There are evidences that this particular group of antioxidant polyphenolics plays a role in metabolic adjustment to both excessive salinity in non-tolerant genotypes (Ismail et al. 2016) and sub-optimal conditions of hyposalinity in obligatory halophytes (Gavin and Durako 2012). FV can be therefore regarded as the first-line defense component of antioxidant system synthesized under stress.

Major PGRs correlating with the functioning of defense mechanisms are distinct in halophyte and glycophyte

Biometrical and biochemical parameters determined in this study were classified into four groups, relevant to general responses to salinity: growth performance, osmotic adjustment, ion accumulation and antioxidant activity. Changes in these parameters occurring at increased salinity levels were analyzed in terms of their

correlations with the changes in concentrations of endogenous PGRs. This approach revealed main differences in the regulation of defense mechanisms by phytohormones in the studied halophyte and glycophyte.

The glycophyte reactions correlated mostly with the changes in SA and ACC levels, as well as ABA (Fig. 2). These three PGRs are usually listed as main regulators of salinity responses in plants, increasing their osmoregulatory and antioxidant capacity (Cui et al. 2015; Van de Poel and Van Der Straeten 2014; Stetsenko et al. 2015), thus the glycophyte reaction was rather typical in this respect. It is worth mentioning that the contents of SA and ACC were interdependent, and some parameters, especially concerning growth and antioxidant activity, also correlated with both of these regulators. Contrary to that, no correlations were found for the pool of auxins, and very limited for Cyt and GA, suggesting that growth promoters were irrelevant for the glycophyte responses to salt exposure. This was rather unexpected, as accumulation of these PGRs substantially increased in *A. alpinus* under stress. Certainly, a tolerance mechanism based on growth retardation in order to minimize loss and survive harsh saline conditions was not activated in this species (Grigore et al. 2012). Unlike in the glycophyte, in the salt-treated halophyte an important role of growth promoters, Cyt, Aux and GA, was observed, although their contents were lower than in the glycophyte. At the same time, none of the growth inhibitors, such as ABA or ethylene precursor, were found to correlate with the determined parameters. Instead, vast responses of the salt-tolerant species were controlled by changes in the concentrations of benzoic acid and jasmonates and also by an interesting interplay between the pools of gibberellins and jasmonates (Fig. 2). The relationship between JA and GA pools is usually antagonistic in stressed plants (Heinrich et al. 2013; Wasternack and Hause 2013). It was particularly visible among the parameters depicting osmotic adjustment: accumulation of ions and organic osmolytes in *A. tripolium*. Progressive accumulation of osmolytes (Na^+ , Cl^- , Pro, GlyB, polyols and mono- and disaccharides) under salinity positively correlated with GA, and negatively with JA contents. Reverse correlations occurred for the concentrations of K^+ and oligosaccharides, the contents of which decreased under salt treatment. None of the determined parameters correlated with JA pool in our glycophyte. It can be related to ineffective osmotic adjustment in this species, based mainly on glucose and ProB accumulation. A recent

study showed that jasmonate signaling may be crucial for GlyB accumulation (Xu et al. 2018), whereas our results suggest that there is no such correlation when ProB synthesis is considered in salt-sensitive *Aster* species.

Enhanced accumulation of SA has been related to mitigation of salt stress effects in plants (Farhangi-Abriz and Ghassemi-Golezani 2018; Gharbi et al. 2017; Youssef et al. 2018). This compound is involved in the regulation of water loss and osmotic conditions during salt exposure, as confirmed in our study in the glycophyte *A. alpinus*. However, we observed that in the halophytic *A. tripolium* benzoic acid, an intermediate compound in PAL-dependent synthesis pathway (Miura and Tada 2014), rather than SA itself, seemed to play a role in modulating defense reactions under salinity. Indeed, our previous study showed that SA was not involved in salinity response in *A. tripolium* (Wiszniewska et al. 2019). Similar reaction was also reported in a halophyte *P. strombulifera* (Devinar et al. 2013). According to Senaratna et al. (2003), it can be assumed that in *A. tripolium* BA is utilized as a signal molecule that controls activation of defense mechanisms under stress. It can be linked to alteration in SA biosynthesis pathway that results in low SA accumulation and BA amassment (Sawada et al. 2006). Our results support the recent opinion that the blockage of SA synthesis is associated with enhanced salinity tolerance (Llanes et al. 2018). The fact that numerous parameters depicting tolerance mechanisms in the halophyte correlated with the content of BA rather than SA is novel, and these relationships should be further investigated.

Conclusions

Our comparative analysis of the functioning of salinity defense mechanisms in related halophyte and glycophyte *Aster* species revealed that both species activated typical salt stress-protecting mechanisms: osmotic adjustment by ion uptake regulation and synthesis of compatible solutes, as well as counteracting oxidative damage. Effective interplay of these mechanisms enabled plant growth under progressive salinity. In the halophytic *A. tripolium*, metabolic modifications became apparent at moderate and high salt concentrations, suggesting that low salinity was not perceived as a stress factor. On the other hand, in the glycophytic *A. alpinus* defense reactions were activated at all salinity levels. As

revealed by the metabolite profiling, the detailed pattern of observed responses was species-specific, and was manifested by synthesis, accumulation or activity of distinct compounds in order to achieve the protective effect. This was particularly visible in the case of osmoprotectant synthesis. Our study linked these differences to altered phytohormonal regulation in salt-tolerant and salt-sensitive plants. The responses of the glycophyte were mostly controlled by changes in the level of endogenous stress hormones: ABA and ethylene precursor ACC, aided also by SA. The halophyte reactions involved changes in the content of plant growth promoters (particularly Aux, but also Cyt and GA), JA and BA. Our study demonstrated that salt tolerance in the studied *Aster* plants is conveyed by benzoic acid rather than salicylic acid and that elevated SA content may even suppress stress tolerance. An important element of salt tolerance trait is also JA-GA network that influences the intensity of particular defense responses. The metabolite profiling also identified features possibly accounting for the glycophyte *A. alpinus* potential to survive abiotic stress conditions. The study reveals new aspects of internal phytohormonal regulation of plant response to salt excess and enables further insight into extremophyte biology.

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Declarations

Conflict of interests Authors declare no conflict of interests.

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