Journal of Plant Growth Regulation
https://doi.org/10.1007/s00344-024-11558-7

Drought- and Salt-Tolerant Populations of the Xero-Halophyte Mediterranean Shrub *Atriplex halimus* L. Exhibit Contrasting Proline and Glycinebetaine Metabolism

Lydia Casasni[1,2] · Cherifa Chaouia[1] · Juan-Pablo Martínez[3] · Muriel Quinet[2] · Stanley Lutts[2]

Received: 7 February 2024 / Accepted: 31 October 2024

Abstract

To determine the effects of ionic and nonionic osmotic stresses, seedlings from two populations of the xero-halophyte species *Atriplex halimus* L. Djelfa (salt-affected site) and Tamanrasset (non-saline arid area) were exposed for 10 days in nutrient solution to NaCl (100 or 300 mM) or to polyethylene glycol (PEG 6000; 50 or 100 g L^{-1}). Ions, proline and glycinebetaine were analyzed in relation to osmotic potential. Enzymes activities involved in proline or glycinebetaine synthesis were compared to the corresponding genes expression. Seedlings from Djelfa were more resistant to NaCl while those from Tamanrasset were more resistant to PEG. Salt-resistance in Djelfa was associated with greater Na^+ accumulation and the maintenance of K^+ concentration. Both ions significantly contributed to osmotic adjustment in salt-stress conditions. Proline accumulated mainly in response to nonionic water stress while glycinebetaine accumulated preferentially in response to salinity. Proline accumulation resulted from a stress-induced stimulation of Δ^1-pyrroline-5-carboxylate reductase and ornithine δ-aminotransferase activities in Tamanrasset and from an inhibition of proline dehydrogenase activity in Djelfa. Glycinebetaine accumulation in Djelfa was associated to an increase in betaine aldehyde dehydrogenase activity while Tamarasset produced the glycinebetaine precursor choline in limiting amounts. Although genes coding for these enzymes were upregulated in most cases, there was a poor correlation between gene expression and enzyme activities suggesting important posttranscriptional regulation. The gene coding for proline/glycinebetaine transporter ProT was upregulated indicating a role of long-distance transport of osmotic compounds in plant resistance to environmental constraints.

Keywords *Atriplex halimus* · Glycinebetaine · Halophyte · Osmotic stress · Proline · Salinity

Introduction

Drought and salt stress are the two major abiotic constraints limiting agricultural productivity throughout the world. Drought frequencies and intensities are expected to increase in the future as consequence of global warming (Haile et al. 2020). According to Khostavichemar et al. (2023), one rapid environmental process in drylands is soil salinization due to the accumulation of soluble water-soluble salts caused by rainfall deficit hindering leaching into the subsoil. Drought increase and soil salinization will have an enormous deleterious impact on cultivated land productivity in the coming decades (Song et al. 2023).

Salinity induces a double constraint on plants. The first one is related to the decrease in the soil water potential compromising plant water uptake, thus inducing a « physiological drought» at the plant level. The second constraint is due to the progressive accumulation of toxic

Handling Editor: Nudrat Aisha Akram

✉ Stanley Lutts
stanley.lutts@uclouvain.be

[1] Laboratoire de Biotechnologie des Productions Végétales, Département de Biotechnologie et Agroécologie, Faculté des Sciences de la Nature et de la Vie, Université de Blida 1, Ouled Yaïch, Algeria

[2] Groupe de Recherche en Physiologie végétale, Earth and Life Institute – Agronomy (ELI-A), Université catholique de Louvain, 5 (Bte 7.07.13) Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

[3] Instituto de Investigaciones Agropecuarias (INIA – Rayentué), Av. Salamanaca s/n, Sector Los Choapinos, Rengo, Chile

Published online: 18 November 2024

ions (mainly Na^+ and excess Cl^-) in the plant tissues (Atta et al. 2023; Fu and Yang 2023). Discriminating between the impacts of osmotic and ionic components of salt stress is a challenging task. With the noticeable exception of *Chenopodium quinoa* and *Amaranthus* sp, cultivated plants are rather sensitive to both drought and salinity (Dietz et al. 2021). In contrast, wild xero-halophyte species developed mechanisms to tolerate these constraints and thrive in these harsh environments (Chen et al. 2023; Mann et al. 2023). Among these mechanisms, osmotic adjustment implies a decrease in the internal osmotic potential (Ψ_s) to maintain a favorable water potential (Ψ_w) gradient thus avoiding tissues dehydration. Toxic ions in halophytes may be sequestered in vacuoles or released and stored in glands and trichomes covering the leaf surface (Ben Hassine et al. 2009; Smaoui et al. 2011; Guo et al. 2023). Xero-halophyte plant species are thus useful plants helping us to identify key physiological characters that should be considered in breeding of cultivated plants (Pérez-Romero et al. 2020; Rahman et al. 2021; Chen et al. 2023). They also constitute a valuable material for ecological restoration of dry or salt areas (Le Houérou 2000). Litalien and Zeeb (2020) even recently discussed implementation strategies that could drive economic feasibility of using halophytes to extract salt excess from salinized substrate and thus mitigate the salinization process.

Proline, glycinebetaine (GB) and soluble sugars (TSS) are reported as efficient osmocompatible solutes. Proline biosynthesis is initiated in the cytosol. The first step consists in phosphorylation and reduction of glutamate by Δ^1-pyrroline-5-carboxylate synthetase (P5CS; EC 1.2.1.41) using NADPH as a cofactor to form glutamate semialdehyde (GSA) which is then non-enzymatically converted in pyrroline-5-carboxylate (P5C). P5C is reduced to proline by P5C reductase (P5CR; EC 1.5.1.2). In mitochondria, ornithine δ-aminotransferase (OAT; EC 2.6.1.13) contributes to the formation of GSA and glutamate by transferring the ornithine δ-amino group to the α-ketoglutarate (Zheng et al. 2021; Ghosh et al. 2022; Alvarez et al. 2022). Proline may be recycled after stress relief through action of proline dehydrogenase (PDH; EC 1.5.5.2). At the whole plant level, proline may be distributed among organs through the proline transporter ProT (Chen et al. 2016).

While all plant species are able to over-produce proline in response to salt and water stresses, glycinebetaine (GB; *N*,*N*,*N* trimethylglycine) is synthesized only in some families and some important cultivated species such as rice or tomato are unable to accumulate significant concentration of this compound (Chen and Murata 2008; Mansour and Ali 2017; Annunziata et al. 2019). Glycinebetaine is synthesized in the chloroplast by a two steps oxidation of choline. The first step is catalyzed by choline monooxygenase (CMO; EC 2.4.15.7) which converts choline to betaine aldehyde. This Rieske-type iron-sulfur and ferredoxin dependent enzyme is often considered as the rate-limiting step for GB synthesis (Wang and Showalter 2004; Tsutsumi et al. 2015). The second step is catalyzed by betaine aldehyde dehydrogenase (BADH; EC 1.2.1.8) which converts betaine aldehyde to GB (Kurepin et al. 2015).

Although proline and GB are able to reduce internal osmotic potential to allow osmotic adjustment avoiding tissue dehydration, they also assume a wide range of other protective functions. GB directly interacts with chloroplast structure and protects photosystem II in response to environmental stress (Huang et al. 2020). It also contributes to the maintenance of reduced to oxidized glutathione ratio, increased antioxidant activity, net photosynthesis and stomatal conductance in harsh conditions (Chen and Murata 2008; Mansour and Ali 2017; Annunziata et al. 2019; Bai et al. 2022). Proline acts as a signal molecule, assumes important antioxidant properties, contributes to the protection of enzymes and numerous cellular structures and the proline cycle acts as a reducing equivalent shuttle (Zheng et al. 2021; Alvarez et al. 2022; Ghosh et al. 2022; Raza et al. 2023; Zulfiqar and Ashraf 2023). It is therefore not surprising that these compounds also accumulate in response to ion toxicities that do not imply a direct osmotic constraint such as heavy metals or arsenic which are toxic at low concentrations (Lutts et al. 2004; Ali et al. 2020a; Zhou et al. 2019; Ghosh et al. 2022; Spormann et al. 2023).

The *Atriplex* genus belongs to the Amaranthaceae family and contains numerous xero-halophyte species able to cope with extremely dry and/or salted areas. Adaptation to such harsh environments has been related to the capacity of those species to synthesize both proline and GB (Le Houérou 2000; Jia et al. 2002; Belkheiri and Mulas 2013; Pan et al. 2016). Genes coding for CMO were identified in *Atriplex nummularia* (Tabuchi et al. 2005) and *A. gmelini* (Tsutsumi et al. 2015). CMO gene from *A. hortensis* was used to confer chilling resistance in cotton (Wang et al. 2019). Similarly, BADH gene from *A. hortensis* (Jia et al. 2002; Fu et al. 2011), *A. canescens* (Qin et al. 2017; Ali et al. 2020a) and *A. micrantha* (Di et al. 2015) conferred salinity or drought resistance in numerous crop species unable to produce GB.

Atriplex halimus L. is a perennial halophyte shrub growing naturally throughout the Mediterranean basin. It exhibits a C4-type photosynthesis and is extremely tolerant to drought and salinity (Walker et al. 2014). In its natural environment, it produces edible biomass that is grazed by cattle and sheeps when herbaceous vegetation is not anymore available at the end of the dry season (Otal et al. 2010; Alotibi et al. 2023). It was also recently reported that leaf extracts of *A. halimus* inhibit the activity of human tumor cell lines (Elbouzidi et al. 2022) or act as biostimulant to increase faba bean plant growth (Ennoury et al. 2023).

Although Na^+ was reportedly contributing to drought resistance in *A. halimus* (Martínez et al. 2005; Nada et al. 2022), this species behaves differently when exposed to drought or salt stress (Ben Hassine and Lutts 2010; Nemat-Alla et al. 2011, 2012; Khedr et al. 2011) and it thus constitutes an interesting material to discriminate between the ionic and the osmotic components of salt stress. This species also displays a high level of variability (Haddioui and Baaziz 2001; Walker et al. 2005; Ortíz-Dorda et al. 2005). It is therefore expected that populations adapted to non-saline xeric habitat exhibit physiological properties different from population adapted to coastal saline areas (Ben Hassine et al. 2010). Few data are available for osmotic adjustment (Ben Hassine et al. 2008), salt excretion capacities (Ben Hassine et al. 2009) and antioxidative properties (Bouchenak et al. 2012). To the best of our knowledge, no data are available regarding the impact of salt or water stress on gene expression and enzyme activities controlling proline and GB synthesis in distinct populations of *A. halimus* adapted to contrasting habitats. The present study was thus undertaken to compare the behavior of a population adapted to saline soil and an inland population adapted to dry conditions. Plants were exposed for 10 days to iso-osmotic concentrations of NaCl and polyethylene glycol (PEG) at two intensities. Proline and glycinebetaine contents, proline and GB-metabolizing enzyme activities and expression of genes coding for these enzymes were analyzed.

Materials and Methods

Plant Material

Seeds of *Atriplex halimus* subsp. *schweinfurthii* were collected on two distinct sites (Fig. 1). Djelfa (34° 40′ 00″ N; 3° 15′ 00″ E) is a semi-arid temperate area with a mean annual rainfall of 267 mm and a mean temperature of 16.85 °C. Considered soil was extremely saline (electrical conductivity (EC) = 17.16 ± 0.41 dS m^{-1}) with a pH of 6.66 ± 0.20 and an organic matter content of 1.01 ± 0.11%. Tamanrasset (Tam; 22° 47′ 13″ N; 5° 31′ 38″ E) was located in the south of Algeria in a warm desertic area, with a mean annual rainfall of 43 mm and a mean annual temperature of 25.3 °C with maximal values close to 40 °C. Plants were growing on a non-saline soil (EC < 1.0 dS m^{-1}), pH 7.51 ± 0.16 and a low organic matter content (0.53 ± 0.04%) (Casasni 2021). On each site, mature seeds were harvested on ten randomly chosen plants and pooled. Seeds were stored at 4 °C before final germination.

Fig. 1 Geographical location of harvesting sites for the two *Atriplex halimus* populations. Djelfa is harvested on saline soil in the El Mesrane region and Tamanrasset in an arid zone and on non-saline soil in the Hoggar zone

Cultural Conditions

Seeds were sown in plastic jars containing a sandy non-saline soil regularly moistened with deionized water in a growth chamber (25 °C, photosynthetic active radiation (PAR) 170 µmol m^{-2} s^{-1}; 16 h day-light photoperiod) in Louvain-la-Neuve (UCLouvain; Belgium). After 5 weeks, seedlings of uniform size were transferred in greenhouse to 2 L tanks of half-strength Hoagland-modified nutrient solution containing (in mM) 5 KNO_3, 1 $NH_4H_2PO_4$, 0.5 $MgSO_4$, 5.5 $Ca(NO_3)_2$ and (in µM) 25 KCl, 10 H_3BO_3, 1 $MnSO_4$, 1 $ZnSO_4$, 0.25 $CuSO_4$, 10 Na_2MoO_4 and 50 mg L^{-1} FeEDTA. Plants (8 per tank) were fixed on polystyrene plates at a mean distance of 6 cm. Temperature was 26 °C during the day and 21 °C during the night, with a mean PAR of 280 µmol m^{-2} s^{-1} provided by natural light supplemented with LED LumiGrow lights (650W; red-blue). Relative humidity oscillated between 47.3 and 61.7% during the time course of experiment. Solutions were renewed each week and were permanently aerated by an EHEIM compact ON300 pump.

Stress treatments were applied 10 days after transfer to nutrient solution. Two types of stress were considered: salt stress (NaCl) and nonionic osmotic stress (polyethylene glycol (PEG) 6000). Two stress intensities were applied: mild stress (NaCl 100 mM (EC = 7.12 dS m^{-1}) or PEG 50 g L^{-1}; both solutions having a $\Psi s = -0.64 \pm 0.03$ MPa) and

a severe stress (NaCl 300 mM (EC 19.6 dS m^{-1}) and PEG 100 g L^{-1}; both solutions with a $\Psi s = -1.87 \pm 0.04$ MPa). Each treatment (type of stress × intensity) involved 5 tanks (40 plants) for each population.

Plant Growth, Water Content and Osmotic Potential

After 10 days of stress, six randomly chosen plants per treatment were harvested and roots were rinsed for 30 s in deionized water to remove ions from the free spaces. Roots, stems and leaves were separated and each type of organ was individually weighed. Samples were then incubated in an oven at 72 °C for 48 h until reaching constant weight: dry weight (DW) was estimated for each sample and dry matter will be subsequently used for mineral analysis (see below).

Osmotic potential (Ψs) was estimated on another set of plants for roots and leaves (considering for each plant two leaves located in the middle part of the stem and already present at the time of stress imposition). Tissular sap was extracted for each type of organ by the centrifugation method according to Bajji et al. (2000). Osmolarity of the extracted sap was measured by a vapour pressure osmometer (Wescor 5500) and converted to osmotic potential according to the Vant'Hof equation.

Chlorophyll and Abscisic Acid Quantification

Chlorophyll (*Chl a* and *Chl b*) and total carotenoid (xanthophyll + β-carotene) concentrations were estimated after grinding 100 mg leaf fresh matter in cold acetone in a pre-chilled mortar. The mixture was filtered and the volume was adjusted to 10 mL with cold acetone. Absorbance of the extract was read at 663.2, 648.8 and 470 nm using a UV-1800 spectrophotometer (Shimadzu, s'Hertogenbosch, The Netherlands). Pigments concentrations were then calculated according to Lichtenthaler (1987).

Abscisic acid (ABA) was extracted according to Saneoka et al. (2001). Tissue samples were ground to a fine powder in 80% acetone (pH 2.8). The solution was then filtered and the residue was extracted twice more with 80% acetone. The homogenate was centrifuged at 2800×*g* during 10 min. After evaporation of the acetone at 40 °C *in vacuo*, the aqueous phase was diluted in TBST buffer (6 g Tris, 0.2 g $MgCl_2$, 8.7. g NaCl, 0.5 mL Tween 20, 0.1 g NaN_3 L^{-1} pH 7.5); aliquots were passed through C_{18} Sep Paks (Waters) and eluted with methanol. The methanol was evaporated to dryness and the residue was dissolved in TBST. Abscisic acid was estimated by an Agrisera abscisic acid ELISA quantification kit (product n° AS20 4392): 50 µL of Antibody and 100 µL of HRP-conjugate were added and samples were incubated at 37 °C during 30 min. Reaction was arrested by adding 50 µM stop solution according to the manufacturer instruction. Optical density was read at 450 nm; ABA was estimated using an ABA calibration curve.

Mineral Content

For each sample, *c.a.* 50 mg dry matter were digested in 4 mL of 0.5% nitric acid at 80 °C. After complete evaporation, residues were dissolved with HNO_3 (68%) + HCl_{cc} (1:3 v/v) and incubated under gentle agitation of 100 rpm for 48 h. The solution was then filtered with Whatman 70 mm Grade 1 and the filtrate was used to determine Na^+ and K^+ concentration by flame emission with an Atomic Absorption Spectrometer (Thermo Scientific S series AAS4 model). Anions were extracted according to Hamrouni et al. (2011). Chloride (Cl^-) concentrations were determined by liquid chromatography (HPLC-Dinex ICS2000, Dionex Corporation, Sunnyvala, California USA) using an AS15/AG15 columns/precolumn system and KOH 10–38 mM as eluant for 45 min.

Proline, Δ^1-Pyrroline-5-carboxylate, Choline, Glycinebetaine and Total Soluble Sugars Concentration

Proline, GB and TSS concentrations were estimated in roots and leaves on six plants per treatment after 10 days of stress exposure.

Proline was quantified according to Bates et al. (1973) on 200 mg of frozen fresh ground material. Proline was extracted in 3% (w/v) sulfosalicylic acid with incubation during 30 min at 70 °C. After filtration (Whatman, 11 µm), 2 mL of ninhydrin solution (1.25 g of ninhydrin in 30 mL glacial acetic acid and 20 mL of H_3PO_4 6 M) were added to the same volume of supernatant and kept at 100 °C for 1 h. Reaction was stopped on ice, then 2 mL toluene were added. Absorbance was read at 520 nm in a quartz cuvette after strong vortexing of the samples. Proline concentration was calculated on the basis of a standard curve established with L-proline (Sigma Aldrich chemicals).

P5C concentration was estimated by the method of Mezl and Knox (1976) based on the formation of 2,4-dinitrophenolhydrazone issued from periodate oxidation of DL-hydroxylysine with slight modification according to La et al. (2020): fresh material was extracted in 3% sulfosalycilic acid containing 2.5 g L^{-1} $CaCl_2$ and centrifuged at 13,000×*g* during 10 min at 4 °C. The supernatants were mixed with 10 mM of 2-aminobenzaldehyde dissolved in 40% ethanol and incubated at 37 °C during 2 h. The absorbance was measured at 440 nm and concentrations were estimated using an extinction coefficient of 2.58 mM^{-1} cm^{-1}.

Table 1 Shoot and root fresh weights (FW, g), water content (WC, %) and osmotic potential (Ψs, MPa) in seedlings of *Atriplex halimus* L. originating from the salt-affected area (Djelfa) or the arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1})

Treatment	Population	FW (g)		WC (%)		Ψs, (MPa)	
		Shoot	Root	Shoot	Root	Shoot	Root
Control	Djelfa	3.56 ± 0.12 d	1.17 ± 0.08 cd	83.2 ± 0.4 e	92.7 ± 0.9 e	− 1.14 ± 0.07 a	− 0.63 ± 0.03 a
	Tam	3.41 ± 0.17 d	1.23 ± 0.09 d	82.8 ± 0.5 d	93.1 ± 0.8 e	− 1.23 ± 0.11 b	− 0.57 ± 0.05 a
Mild stress							
NaCl 100 mM	Djelfa	4.22 ± 0.21 f	1.52 ± 0.05 e	84.5 ± 0.4 e	90.4 ± 1.2 de	− 2.54 ± 0.06 de	− 1.48 ± 0.01 d
	Tam	3.81 ± 0.15 e	1.26 ± 0.14 d	83.1 ± 0.3 e	88.4 ± 0.3 d	− 2.32 ± 0.09 d	− 1.32 ± 0.04 c
PEG 50 g L^{-1}	Djelfa	3.01 ± 0.27 bc	1.01 ± 0.03 b	80.5 ± 0.2 cd	87.2 ± 0.7 cd	− 2.41 ± 0.10 d	− 0.92 ± 0.05 b
	Tam	3.46 ± 0.18 d	1.32 ± 0.11 de	82.5 ± 0.7 d	91.3 ± 0.4 e	− 2.66 ± 0.04 e	− 1.38 ± 0.06 c
Severe stress							
NaCl 300 mM	Djelfa	3.25 ± 0.07 c	0.98 ± 0.04 b	79.4 ± 0.9 c	86.5 ± 0.6 c	− 2.64 ± 0.05 e	− 1.88 ± 0.04 e
	Tam	2.94 ± 0.14 b	1.12 ± 0.07 c	78.3 ± 0.4 bc	85.4 ± 0.3 cb	− 2.31 ± 0.08 d	− 1.13 ± 0.07 b
PEG 100 g L^{-1}	Djelfa	2.13 ± 0.11 a	0.71 ± 0.06 a	70.8 ± 1.2 a	81.4 ± 0.2 a	− 2.07 ± 0.10 c	− 1.53 ± 0.11 d
	Tam	3.01 ± 0.18 bc	1.07 ± 0.14 bc	76.3 ± 0.6 b	83.3 ± 0.4 b	− 2.61 ± 0.14 e	− 1.92 ± 0.07 e

Each value is the mean of six replicates ± standard errors. For a given parameter and organ, means with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test

The GB concentration was estimated by the method of Grieve and Grattan (1983) with slight modifications according to Bouchenak et al. (2012). Samples were ground in the presence of liquid nitrogen until a fine powder was obtained. Powder was stirred in 20 mL distilled water and incubated for 24 h, filtered and stored at − 80 °C. Extracts were 1:1 with 2 M H_2SO_4 and aliquots (0.5 mL) were transferred to centrifuge tubes and cooled on ice for 1 h. KI-I_2 reagent was prepared by dissolving 15.7 g iodine and 20 g KI in 100 mL water. Cold KI-I_2 (200 µL) was added to centrifuge tubes containing sample extract and thoroughly vortexed. Samples were then incubated at 4 °C during 16 h and then centrifuged at 8000×*g* for 15 min at 4 °C. The obtained crystals were dissolved in 1,2-dichloroethane and the absorbance was measured at 365 nm. Standard curve was established using GB (SigmaAldrich) dissolved in 1 M H_2SO_4. Choline was extracted from 2 g fresh samples after homogenization in 10 mL cold extraction reagent (metha nol:chloroform:water = 12:5:3 v/v). After adding 2 mL of water, the mixture was centrifuged at 3000×*g* for 10 min. After evaporation to dryness *in vacuo*, the dried samples were redissolved in 3 mL of 2-propanol containing 3% water. The choline concentration was then quantified by the triiodide/activated charcoal/molybdenum blue method after resuspension of choline iodide in ethylene dichloride according to Zimmerman and Ibrahim (2018).

Soluble sugars were extracted in 80% ethanol from finely ground powder. Samples were centrifuged for 10 min at 8000×*g* and total soluble sugars concentrations were determined in the supernatant using the anthrone method by reading absorbance at 625 nm according to Yemm and Willis (1954). A calibration curve was established using glucose as standard.

Enzyme Activities Determination

P5CR and PDH were extracted from frozen roots and leaves in a buffer containing 50 mM Tris–HCl (pH 7.6) with 7 mM $MgCl_2$, 0.6 M KCl, 3 mM ethylene diamine tetraacetic acid (EDTA), 1 mM DTT and 5% (w/v) insoluble polyvinylpyrrolidone (PVP). The obtained homogenates were filtered through two layers of Miracloth ((# Grade 80 µm) and then centrifuged at 39,000×*g* for 30 min at 4 °C. The collected supernatants were desalted two times on Sephadex G25-medium column equilibrated with 50 mM Tris–HCl (pH 7.6) containing 10% glycerol. OAT was extracted in 100 mM K-Pi buffer (pH 7.9) containing 1 mM EDTA, 15% glycerol and 10 mM β-mercaptoethanol and samples were centrifuged at 23,000×*g* 4 °C for 20 min. Collected supernatants were then treated with $(NH_4)_2SO_4$ at 60% saturation for 45 min and then re-centrifuged at 23,000×*g* for 15 min before desalting on Sephadex G25 column equilibrated with the extraction buffer.

P5CR activity was estimated in a reaction buffer consisting in 50 mM Tris–HCl buffer (pH 7.0) with 1 mM dithiothreitol (DTT), 1 mM P5C and 0.25 mM NADH; the reactions started with the addition of 0.1 mL crude extract and the decrease in absorbance was monitored at 340 nm. PDH activity was quantified by monitoring the $NADP^+$ reduction at 340 nm in 0.15 M Na_2CO_3 buffer (pH 10.3)

containing 15 mM proline and 1.5 mM $NADP^+$ according to Lutts et al. (1999). OAT was assayed in 0.2 M Tris-KOH (pH 8.0) containing 5 mM ornithine, 10 mM α-ketoglutarate and 0.25 mM NADH in a volume of 2.0 mL; the decrease in absorbance was monitored at 340 nm after initiating the reaction with addition of 0.2 mL of the enzyme extract.

BADH was extracted in 0.1 M Tricine-KOH, pH 8.5 1 mM EDTA, 2 mM DTT and 0.6 M sucrose according to Weretilnyk and Hanson (1986). The reactions were carried out in a final volume of 1 mL containing 50 mM HEPES–KOH (pH 8.0), 10 mM EDTA, 1 mM NAD^+, 1 mM betaine aldehyde and 1 mg protein extract. BADH activity was followed by the betaine aldehyde-specific reduction of NAD^+ at 22 °C.

For each enzyme activities (EA) determination and each treatment (population × type of stress × stress intensity), measurements were performed on four samples each containing three plants. The relative modification of enzyme activities (RM_{EA}) was defined for each enzyme and type of stress (nature of the stressor × stress intensity) as $EA_{stress}/EA_{controls}$. All EA were expressed on a protein basis. Protein concentration was estimated according to Bradford (1976).

RNA Extraction and Semi-quantitative Gene Expression

Total RNA was isolated from leaves and roots as previously described (Quinet et al. 2014) with slight modifications. All steps were performed at 4 °C. Approximately 500 mg of fresh matter were ground in liquid nitrogen and transferred into 7 mL extraction buffer (1 M Tris–HCl, 0.5 M EDTA, 5 M NaCl, 2% (v/v) β-mercaptoethanol, pH 8) previously preheated at 65 °C. The mixture was incubated at 65 °C for 15 min in a water bath and agitated every minute. After centrifugation at 4500×*g* for 20 min at 4 °C, 7 mL of chloroform/isoamylalcohol (24:1) were added to the

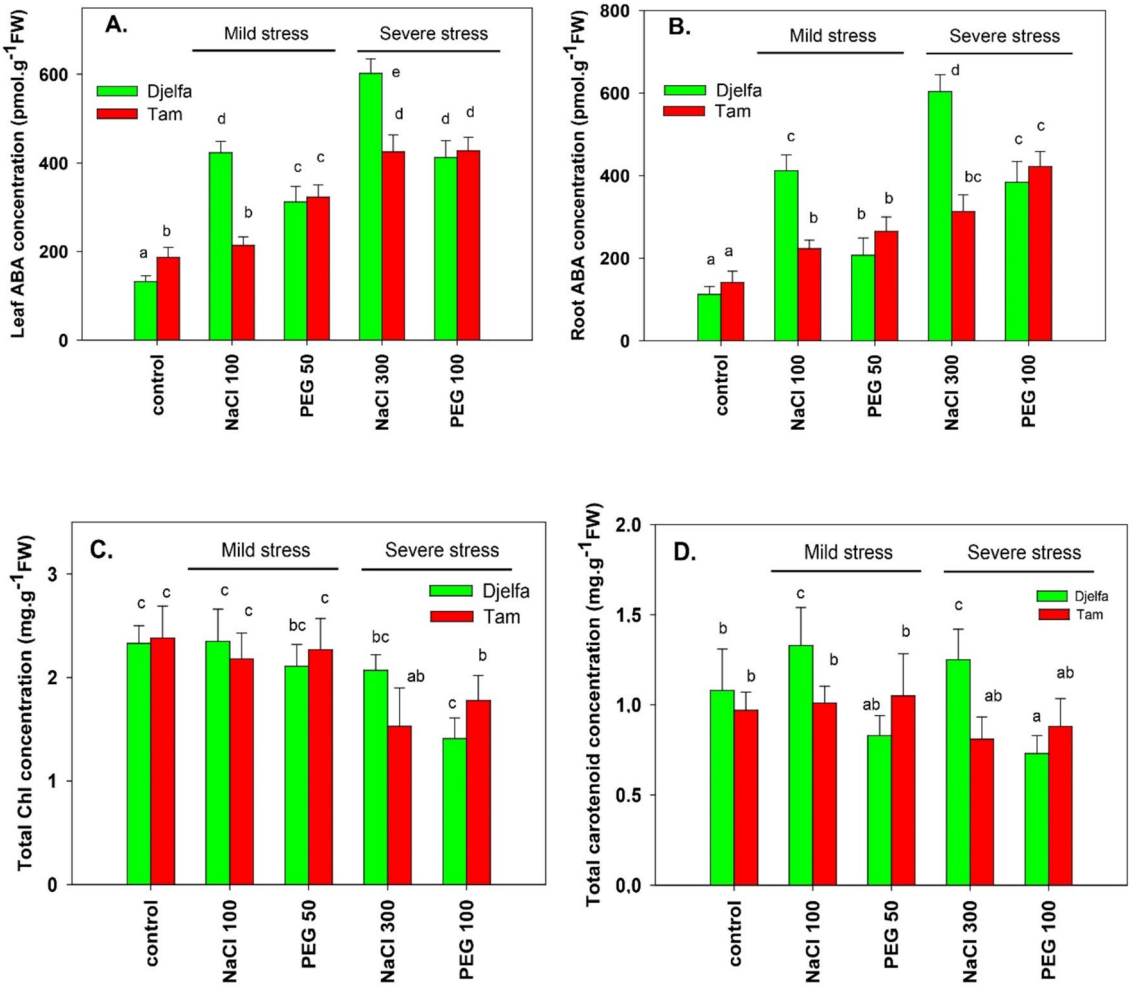


Fig. 2 Leaf and roots abscisic acid concentration (ABA), total chlorophyll concentration (*Chl*) and carotenoid concentration in seedlings of *Atriplex halimus* originating from the salt-affected area (Djelfa) or the arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1}). Each value is the mean of six replicates ± standard errors. Bars with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test. Leaf and roots abscisic acid concentration (ABA) (**A** and **B**), total chlorophyll concentration (**C**) and carotenoid concentration (**D**)

Table 2 Sodium (Na^+), potassium (K^+) and chloride (Cl^-) in seedlings of *Atriplex halimus* L. originating from the salt-affected area (Djelfa) or the arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1})

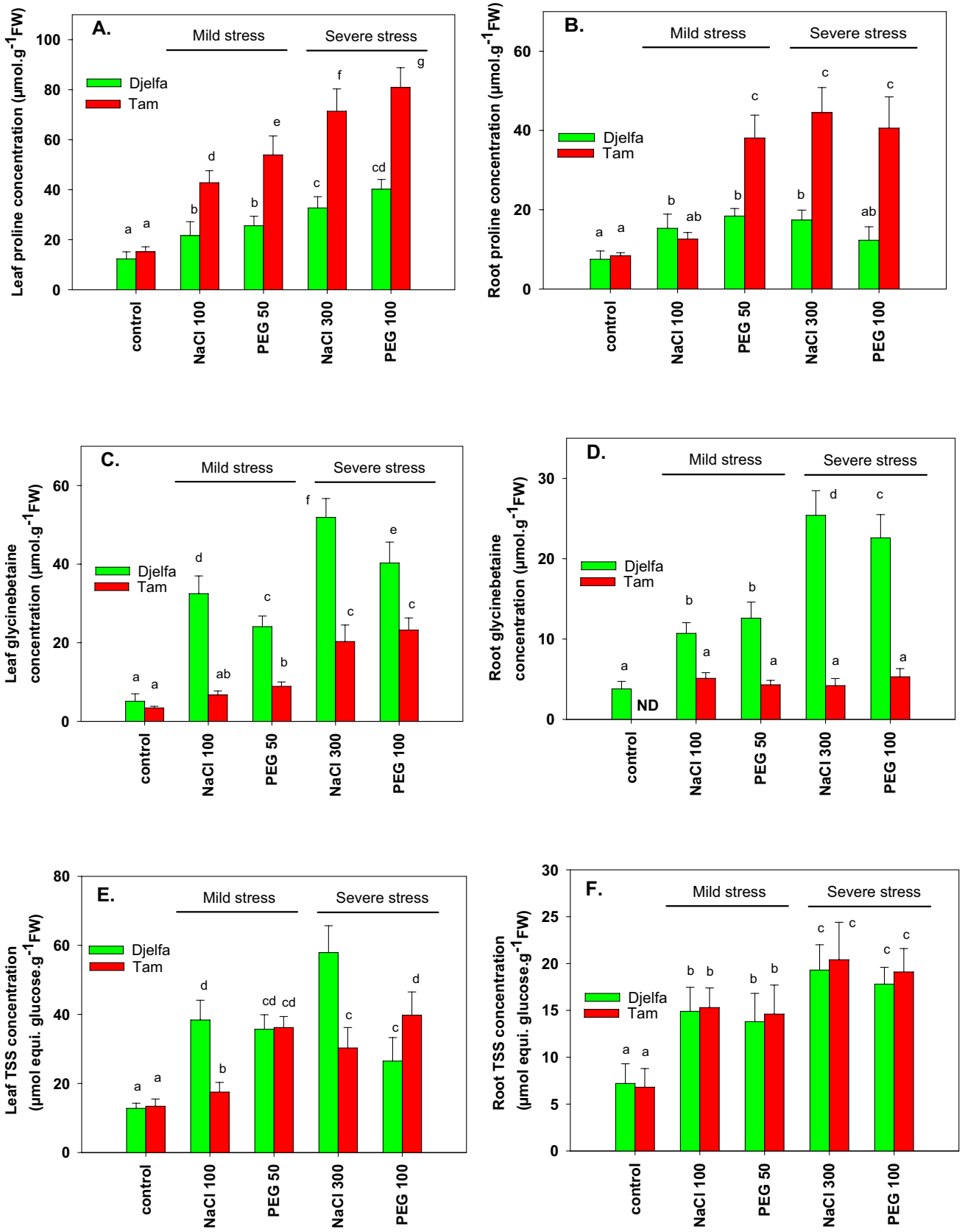
Treatment	Population	Na^+ (µmol g^{-1} DW)		K^+ (µmol g^{-1} DW)		Cl^- (µmol g^{-1} DW)	
		Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	Djelfa	0.07 ± 0.01 a	0.12 ± 0.02 a	1.94 ± 0.14 cd	2.58 ± 0.17 b	0.21 ± 0.03 a	0.15 ± 0.01 b
	Tam	0.05 ± 0.01 a	0.10 ± 0.02 a	1.81 ± 0.12 c	2.42 ± 0.11 b	0.30 ± 0.01 ab	0.23 ± 0.02 c
Mild stress							
NaCl 100 mM	Djelfa	1.13 ± 0.12 d	1.57 ± 0.08 c	1.89 ± 0.13 c	2.47 ± 0.07 b	1.03 ± 0.08 e	1.99 ± 0.09 g
	Tam	0.88 ± 0.08 c	1.63 ± 0.11c	1.42 ± 0.07 a	3.07 ± 0.09 c	1.68 ± 0.12 f	1.62 ± 0.10 f
PEG 50 g L^{-1}	Djelfa	0.08 ± 0.01 a	0.10 ± 0.01 a	1.53 ± 0.10 ab	2.63 ± 0.06 b	0.25 ± 0.03 a	0.14 ± 0.01 b
	Tam	0.15 ± 0.02 b	0.21 ± 0.02 b	2.26 ± 0.17 d	3.26 ± 0.12 cd	0.43 ± 0.02 c	0.07 ± 0.02 a
Severe stress							
NaCl 300 mM	Djelfa	1.89 ± 0.17 e	2.37 ± 0.14 e	1.74 ± 0.15 bc	2.88 ± 0.12 bc	2.46 ± 0.25 g	3.01 ± 0.15 h
	Tam	1.25 ± 0.09 d	1.89 ± 0.09 d	1.38 ± 0.09 a	1.57 ± 0.09 a	2.53 ± 0.16 g	3.12 ± 0.17 h
PEG 100 g L^{-1}	Djelfa	0.13 ± 0.02 ab	0.11 ± 0.01 a	1.60 ± 0.14 b	2.94 ± 0.12 c	0.32 ± 0.05 b	0.43 ± 0.04 e
	Tam	0.19 ± 0.01b	0.22 ± 0.03 b	2.37 ± 0.21 d	3.33 ± 0.11 d	0.57 ± 0.10 d	0.21 ± 0.03 c

Each value is the mean of six replicates ± standard errors. For a given parameter and organ, means with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test

supernatant and the mixture was centrifuged at 4500×*g* for 20 min. This operation was repeated three times before transfer of the aqueous phase in a new tube and addition of 0.1 volume of Na-acetate 3 M pH 5.2 and 0.6 volume of isopropanol. After precipitation at − 80 °C for 30 min and centrifugation at 8400×*g* for 30 min, the pellet was dried and then re-suspended in 1 mL of Tris–EDTA buffer pH 7.5. Total RNA was then precipitated overnight after addition of 300 µL of 10 M LiCl. After centrifugation (18,000×*g* during 30 min), the pellet was washed in 500 µL 70% ethanol and re-suspended in DEPC-water. RNA concentration was quantified using the NanoDrop ND-1000 (Isogen Life Science, De Meern, The Netherlands) by reading absorbance at 260 nm and purity was evaluated with 260/280 and 230/260 ratios.

DNase treatments were realized using RQ1 RNase-free DNase (Promega, Leiden, The Netherlands) according to the manufacturer's instructions. Reverse Transcription was performed with 1 µg of total RNA using the GoScriptTM Reverse Transcription Mix, Oligo (dT) Protocol Kit (Promega Benelux bv., Leiden, The Netherlands) by following the manufacturer's instructions. Amplifications were conducted using GoTaq DNA polymerase (Promega Benelux b.v., Leiden, The Netherlands) with 500 ng of cDNA and specific primers for *Actin* and target genes *BADH*, *CMO*, *P5CS1*, *P5CR*, *ProT* and *OAT* (Table S1). Degenerate primers for the studied genes were designed to recognize the conserved motifs resulting from the alignment of the characterized protein sequences from close-related species from Amaranthaceae found in NCBI and ExPASy databases. When the sequences were not described in the *Atriplex* genus, homologs were identified using BLAST against *Beta vulgaris*, *Spinacea oleracea* and *Chenopodium rubrum* in National Center for Biotechnology Information (NCBI) and alignment was performed to provide reverse and forward consensus primers for considered genes (see Fig. S1). After an initial denaturation step at 95 °C for 2 min, each cycle consisted of 30 s at 95 °C, 45 s at an annealing temperature depending on the primer combination and 15 s extension at 72 °C, followed by a final extension of 10 min at 72 °C. The polymerase chain reaction (PCR) products were resolved on agarose gels. At least three independent PCR amplifications were conducted for each gene using the primer pairs, annealing temperatures, and number of cycles presented in Table S1. Expression differences were analyzed by gel densitometry using ImageJ software and expressed as relative values compared to *Actin* expression [Average peak size of target gene/peak size of *Actin*] (Quinet et al. 2014). Gene expression analyses were repeated for three independent cultures and gave similar results.

In order to ensure a direct comparison between EA and gene regulation, RT-PCR was conducted on the same samples previously used for EA measurement. For each gene, the relative modification of transcripts abundance (RM_{TA}) was defined as $TL_{stress}/TL_{controls}$.


A.
Leaf proline concentration (µmol.g-1FW)
Mild stress
Severe stress
Djelfa
Tam
control
NaCl 100
PEG 50
NaCl 300
PEG 100
B.
Root proline concentration (µmol.g-1FW)
C.
Leaf glycinebetaine concentration (µmol.g-1FW)
D.
Root glycinebetaine concentration (µmol.g-1FW)
ND
E.
Leaf TSS concentration (µmol equi. glucose.g-1FW)
F.
Root TSS concentration (µmol equi. glucose.g-1FW)

◂**Fig. 3** Leaves and roots proline (**A** and **B**), glycine betaine (**C** and **D**) and total soluble sugars (TSS; **E** and **F**) in seedlings of *Atriplex halimus* originating from the salt-affected area (Djelfa) or from the non-saline arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1}). Each value is the mean of six replicates ± standard errors. Bars with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test; *ND* not detected

Statistical Treatment of the Data

All statistical analyses were performed with R Software Version 3.6.1 (RDevelopment and C. TEM 2009). The data normality was checked by Shapiro–Wilk tests and homoscedasticity by Levene's tests and data were transformed when required. Differences among treatment were analyzed using two-way or three-way analysis of variance (ANOVA) with population, type of stress and stress intensity as main factors. When significant differences were observed ($P < 0.05$), post hoc Tukey's HSD test was performed using all-pairwise comparisons to compare means.

Results

Detailed results of ANOVA 3 statistical analysis are provided in Table S2.

Plant Growth, Water Content and Osmotic Potential

All plants remained alive until the end of the treatment. At the end of the experiment, seedlings in control conditions from the two populations displayed contrasting height (74.3 ± 1.27 cm for Djelfa and 59.4 ± 3.6 for Tam; $P < 0.05$) but similar number of leaves (13.7 ± 1.4 for Djelfa and 14.2 ± 1.5 for Tam). Treatments had no impact on these parameters, whatever he nature of stress factor or stress intensity (detailed data not shown). In contrast, stress treatment had a strong impact on fresh weight (FW), water content (WC) and Ψs (Table 1 and Table S2). A moderate dose of NaCl (100 mM) increased both shoot and root FW in Djelfa while it increased shoot FW but not root FW in Tam. A mild osmotic stress (PEG 50 g L^{-1}) decreased shoot and root FW in Djelfa but had no impact in Tam. A high NaCl dose (300 mM) decreased FW to a higher extent in Tam than in Djelfa, while an opposite trend was recorded for high PEG (100 g L^{-1}). Djelfa was more affected by high PEG than by high NaCl, while no difference between NaCl and PEG was recorded for Tam.

Shoot WC decreased in response to mild osmotic stress in Djelfa but remained unaffected in Tam. It decreased in both populations exposed to 300 mM NaCl. It exhibited a minimal value in Djelfa exposed to PEG 100 g L^{-1}. Similarly, in the presence of PEG, the root WC was always lower in Djelfa than in Tam. The shoot and root Ψs decreased in response to all stress treatments. In the presence of NaCl, shoot and root Ψs were lower in Djelfa than in Tam while an opposite trend was observed for plants exposed to PEG.

Chlorophyll and Abscisic Acid Quantification

Abscisic acid accumulated in leaves (Fig. 2A) and in roots (Fig. 2B) in response to NaCl or PEG. In response to a mild stress, salt-treated plants from Djelfa accumulated higher concentrations of ABA in leaves and roots than plants from Tam, while both populations behaved similarly in response to PEG 50 g L^{-1}. A similar trend was recorded in response to severe stress (300 mM NaCl or PEG 100 g L^{-1}). As far as plants from Djelfa are concerned, ABA concentration was higher in response to NaCl than in response to PEG, while this was not the case for Tam.

Mild stress had no significant impact on total chlorophyll (Fig. 2C) and did not modify *Chl* a/*Chl* b ratio (detailed data not shown). A high NaCl dose (300 mM NaCl) significantly reduced the total *Chl* concentration in Tam while high PEG concentration decreased the total *Chl* concentration in Djelfa and to a lower extent in Tam. Salinity increased the total carotenoid concentration in Djelfa while high PEG concentration (100 g L^{-1}) decreased it (Fig. 2D).

Mineral Content

Sodium accumulated in all plants exposed to NaCl (Table 2) and a higher leaf Na^+ concentration was recorded for Djelfa than for Tam at both 100 mM and 300 mM NaCl. Although leaf and root Na^+ remained rather low in PEG-treated plants, they significantly increased comparatively to control plants in Tam but not in Djelfa. Leaf K^+ concentration remained unaffected by salt treatments in Djelfa while it significantly decreased in Tam. In contrast, leaf K^+ concentration was always higher in Tam than in Djelfa in PEG-treated plants. In Djelfa, the root K^+ concentration increased in plants exposed to high PEG concentration but remained unchanged in response to other treatments while in Tam, it decreased in plants exposed to 300 mM NaCl but it increased comparatively to controls in all other treatments. Leaf Cl^- concentration was higher in Tam than in Djelfa except at 300 mM NaCl where both populations presented similar values. In contrast, root Cl^- was lower in Tam than in Djelfa except

Table 3 Leaves and roots Δ^1-pyrroline-5-carboxylate (Δ^1-P5C) and choline concentrations in seedlings of *Atriplex halimus* L. originating from the salt-affected area (Djelfa) or the arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1})

Treatment	Population	Δ^1-P5C (µmol g^{-1} FW)		Choline (µmol g^{-1} FW)	
		Leaves	Roots	Leaves	Roots
Control	Djelfa	1.85 ± 0.15 a	1.16 ± 0.11 a	3.21 ± 0.15 a	3.54 ± 0.32 a
	Tam	1.68 ± 0.16 a	1.22 ± 0.11 a	2.80 ± 0.21 a	2.11 ± 0.68 a
Mild stress					
NaCl 100 mM	Djelfa	1.77 ± 0.09 a	1.18 ± 0.15 a	7.94 ± 0.69 b	6.17 ± 0.45 b
	Tam	1.44 ± 0.24 a	1.25 ± 0.09 a	3.55 ± 0.14 a	ND
PEG 50 g L^{-1}	Djelfa	1.69 ± 0.14 a	1.27 ± 0.12 a	6.83 ± 0.54 b	7.13 ± 0.66 b
	Tam	2.81 ± 0.19 b	2.35 ± 0.05 c	3.07 ± 0.11 a	2.34 ± 0.35 a
Severe stress					
NaCl 300 mM	Djelfa	2.11 ± 0.22 a	1.33 ± 0.12 a	10.41 ± 0.19 c	9.58 ± 0.17 c
	Tam	1.79 ± 0.13 a	1.99 ± 0.06 b	5.49 ± 1.25 ab	ND
PEG 100 g L^{-1}	Djelfa	1.67 ± 0.07 a	1.46 ± 0.21 a	9.99 ± 0.36 c	11.87 ± 0.23 d
	Tam	3.01 ± 0.23 b	2.18 ± 0.07 b	4.65 ± 1.14 a	2.63 ± 0.21 a

Each value is the mean of six replicates ± standard errors. For a given parameter and organ, means with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test

ND not detected

once again at 300 mM NaCl which induced similar root Cl^- concentrations for the two populations.

Proline, Δ^1-Pyrroline-5-carboxylate, Choline, Glycinebetaine and Total Soluble Sugars Concentration

After 10 days of stress exposure, proline accumulated to similar concentrations (Fig. 3A, B) in response to NaCl and PEG in Djelfa while Tam displayed a higher proline concentration when exposed to PEG comparatively to NaCl. Proline accumulation was always higher in Tam than in Djelfa, whatever the nature of the stressor (Fig. 3A, B). In all treatments including controls, proline concentration was higher in the leaves than in the roots. The leaf and root P5C concentration (Table 3) increased in seedlings of Tam exposed to PEG, but not in seedlings exposed to NaCl. Leaf P5C concentration Djelfa remained unaffected by the treatments.

Glycinebetaine (Fig. 3C, D) accumulated to higher concentration in Djelfa than in Tam, whatever the nature of the stressor or the stress intensity. The highest leaf GB concentration was recorded in Djelfa exposed to NaCl. In contrast, no difference was recorded between Tam exposed to NaCl or to PEG (Fig. 3C). While root GB concentration was increased more than tenfolds in Djelfa exposed to severe stress intensity, Tam did not accumulate GB at the root level in response to NaCl and PEG (Fig. 3D). Choline concentration (Table 3) was always higher in Djelfa than in Tam. It increased in leaves and roots in Djelfa in response to all treatments and the recorded increase was higher under severe than under mild stress. In contrast, it did not increase in Tam and root choline concentration was even below the detectable limits in Tam seedlings exposed to NaCl.

Leaf total soluble sugars concentration (Fig. 3E) increased in response to salinity and was higher in Djelfa than in Tam for plants exposed to 100 or to 300 mM NaCl. Plants issued from the two populations accumulated similar TSS concentrations in the leaves when exposed to PEG 50 g L^{-1} but plants from Djelfa presented a lower TSS value comparatively to Tam in response to PEG 100 g L^{-1}. At the root level (Fig. 3F), TSS concentration was similar in the two populations and no difference between PEG and NaCl was recorded for a given stress intensity (Fig. 3F).

Enzyme Activities Determination

P5CR activity (Fig. 4A, B) increased in the leaves of stressed plants to a higher extent in Tam seedlings than in Djelfa. For each stress intensity, the recorded increase was especially high in salt-treated seedlings of Tam and the highest P5CR activity was recorded for seedlings exposed to 300 mM NaCl. P5CR activity in roots of Tam seedlings was also higher in response to NaCl than in response to PEG. The leaf OAT activity (Fig. 4C) was higher in Tam than in Djelfa and activity was higher in seedlings exposed to PEG than in those exposed to NaCl. The root OAT activity (Fig. 4D) was also lower in Djelfa than in Tam. In Djelfa, only high PEG concentration induced an increase in OAT activity in the leaves but not in the roots. The root and shoot PDH activities (Fig. 4E, F) were reduced in Djelfa exposed to NaCl and PEG. At the leaf level, the recorded decrease was more marked at the highest stress intensity for both type of stressors and no difference was recorded between NaCl and PEG treatments in Djelfa. The leaf PDH activity was not

affected by the treatments in Tam (except a small decrease recorded in response to PEG 100 at the leaf level) and only marginally decreased at the root level.

BADH activity (Table 4) significantly increased in Djelfa in response to stress: at the leaf level, BADH activity was higher in response to NaCl than in response to PEG. Leaf and root BADH activities were always lower in Tam than in Djelfa. No difference was recorded between the two types of stressor for Tam.

Semi-quantitative Gene Expression

In leaves of Djelfa, the gene coding for P5CS1 (Fig. 5A) was upregulated in response to salinity and also at the highest dose of PEG. In leaves of Tam, the gene was upregulated in response to the highest stress intensity and to a higher extent in response to PEG than in response to NaCl. The expression of this gene at the root level was slightly increased in response to the highest stress intensity only. The expression of *P5CR* (Fig. 5B) in the leaves increased in response to all types of stress and no significant difference was recorded between populations. At the root level, however, *P5CR* gene was upregulated in response to severe stress intensity in Tam. *PDH* gene (Fig. 5C) was upregulated in the leaves of Djelfa exposed to 50 and 100 g L^{-1} PEG, and to a lower extent in the leaves of Tam exposed to 100 and 300 mM NaCl. At the root level, *PDH* expression was inhibited in Tam exposed to 100 mM NaCl but remained unaffected in other treatments. Only a faint signal was recorded for *OAT* (Fig. 5D): this gene expression was similar in both populations and remained unaffected by the stress treatments.

The expression of gene coding for ProT (Fig. 6A) increased in response to a mild stress in the leaves of Djelfa but not in the leaves of Tam. In response to severe stress, both populations exhibited an increase in *ProT* expression which was more marked for Djelfa than for Tam. At the root level, *ProT* expression was increased in Djelfa, the recorded increase being proportional to the stress intensity. In contrast, root *ProT* expression remained unaffected in Tam. Gene coding for CMO (Fig. 6B) was upregulated in the leaves of Djelfa in response to all stressors while it was slightly inhibited in response to stress in leaves of Tam. This gene expression remained low in roots and was not affected by stress.

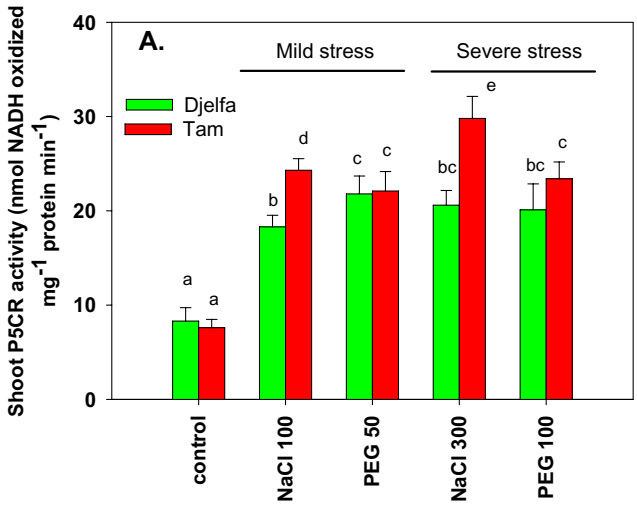
BADH expression (Fig. 6C) was increased in the leaves of Djelfa. As far as Tam is concerned, a moderate upregulation of *BADH* expression was recorded in the leaves in response to PEG only. BADH expression in the roots increased in response to 100 mM NaCl, PEG 50 and PEG 100 in both populations, although to a higher extent in Tam than in Djelfa for moderate stress intensities. In contrast, root *BADH* expression was not affected in response to 300 mM NaCl and was even not detected in the roots of Tam exposed to this treatment.

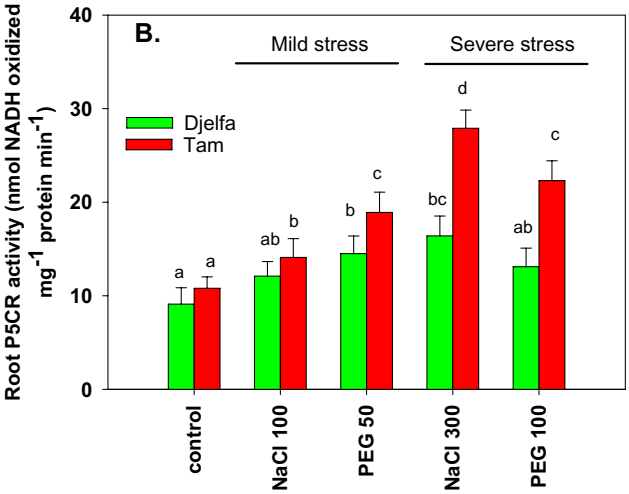
Discussion

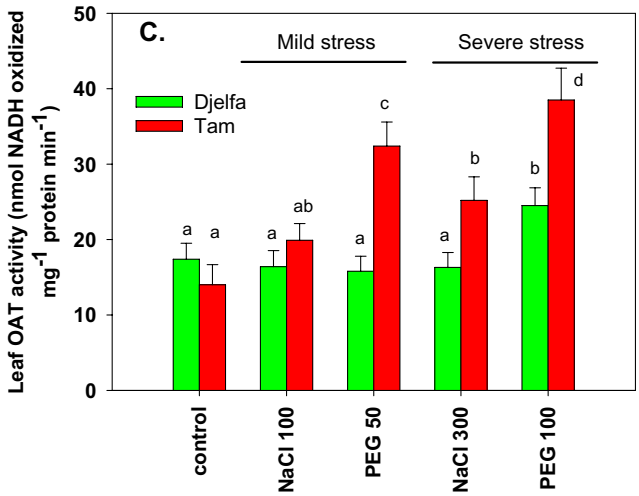
Distinct Populations Displayed Contrasting Levels of Salt and Osmotic Stress Tolerance

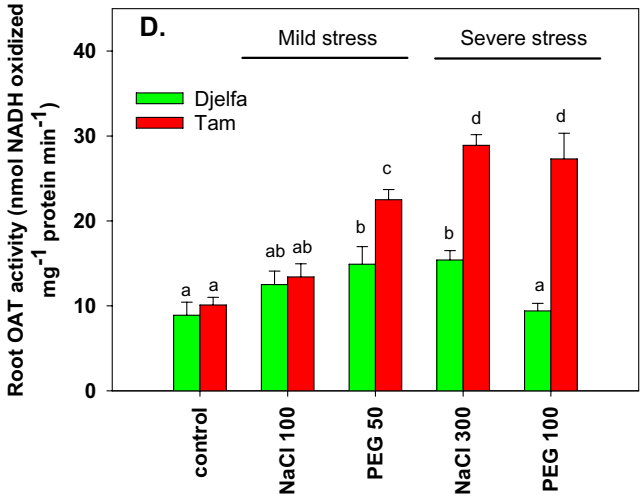
The fact that no mortality occurred at high stress intensities during the time course of the experiment confirmed that *Atriplex halimus* is a well-adapted xero-halophyte plant species able to cope with water and salt stresses (Walker et al. 2014). However, our work provides clear evidences that the two populations behaved differently and were well adapted to their respective habitat. Indeed, Djelfa native to a highly saline area (EC = 17.16 dS m^{-1}) was clearly more tolerant to NaCl than Tam originating from an inland dry and non-saline site as indicated by a better growth maintenance and osmotic adjustment of salt-treated plants of Djelfa comparatively to Tam. A moderate dose of NaCl even stimulated growth in Djelfa, which is typical from halophyte plants (Atta et al. 2023; Fu and Yang 2023) and has been reported in *Atriplex halimus* (Martínez et al. 2005; Nemat-Alla et al. 2011; Nada and Abogadallah 2015) *A. portulacoides* (Benzarti et al. 2014), *A. nummularia* (Silveira et al. 2009; Belkheiri and Mulas 2013) and *A. canescens* (Pan et al. 2016). In the present case, it is noteworthy that such a NaCl-induced growth stimulation was not observed in Tam issued from a non-saline area. Tam, however, was more efficient than Djelfa to face nonionic osmotic stress and this could be related to the fact that this population is issued from an area with quite low and erratic rainfall where drought stress resistance is an important component of plant survival.

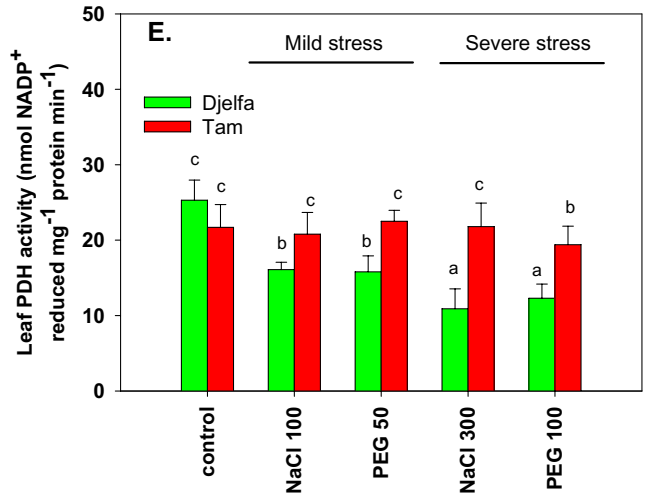
Our data demonstrated that the highest salt-tolerance of Djelfa was not due to a lower accumulation of Na^+: rather, Djelfa behaved as a typical includer which did not restrict Na^+ absorption or translocation and it accumulated higher concentrations of Na^+ than Tam in all organs of salt-treated plants (Table 2). It is tempting to speculate that accumulated Na^+ might contribute to osmotic adjustment as suggested by Pan et al. (2016) in *A. canescens*. This is supported by the fact that Ψ_s of salt-treated Djelfa was significantly lower than Ψ_s values recorded in Tam (Table 1). Since cytosolic Na^+ is detrimental to enzyme activities, even in halophyte plant species, this implies that excess of Na^+ must be sequestered in vacuoles (Pan et al. 2016). Nada and Abogadallah (2015) found that genes coding for NHX1 and H^+-PPase involved in Na^+ tonoplast transport were upregulated by salinity in *A. halimus*. The same authors reported that gene coding for SOS1 involved in Na^+ efflux to surrounding medium at the root level may also be induced by NaCl in some accessions and this could explain the lower Na^+ concentration recorded

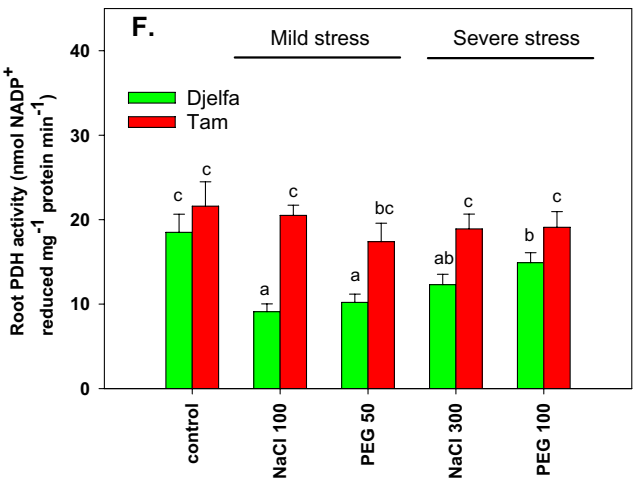

A.
Mild stress
Severe stress
Djelfa
Tam
Shoot P5CR activity (nmol NADH oxidized mg-1 protein min-1)
control
NaCl 100
PEG 50
NaCl 300
PEG 100


B.
Mild stress
Severe stress
Djelfa
Tam
Root P5CR activity (nmol NADH oxidized mg-1 protein min-1)
control
NaCl 100
PEG 50
NaCl 300
PEG 100


C.
Mild stress
Severe stress
Djelfa
Tam
Leaf OAT activity (nmol NADH oxidized mg-1 protein min-1)
control
NaCl 100
PEG 50
NaCl 300
PEG 100


D.
Mild stress
Severe stress
Djelfa
Tam
Root OAT activity (nmol NADH oxidized mg-1 protein min-1)
control
NaCl 100
PEG 50
NaCl 300
PEG 100


E.
Mild stress
Severe stress
Djelfa
Tam
Leaf PDH activity (nmol NADP+ reduced mg-1 protein min-1)
control
NaCl 100
PEG 50
NaCl 300
PEG 100


F.
Mild stress
Severe stress
Djelfa
Tam
Root PDH activity (nmol NADP+ reduced mg-1 protein min-1)
control
NaCl 100
PEG 50
NaCl 300
PEG 100

◂**Fig. 4** Leaves and roots Δ^1-pyrroline-5-carboxylate reductase activity (P5CR; **A** and **B**, in nmol NADH oxidized mg $prot^{-1}$ min^{-1}), ornithine δ-aminotransferase activity (OAT; **C** and **D**, in nmol NADH oxidized mg $prot^{-1}$ min^{-1}) and proline dehydrogenase activity (PDH; **E** and **F**, in nmol ($NADP^+$ reduced mg^{-1} protein min^{-1}) in seedlings of *Atriplex halimus*. originating from the salt-affected area (Djelfa) or from the non-saline arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1}). Each value is the mean of four replicates ± standard errors. Bars with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test

in Tam. An important portion of the absorbed Na^+ may also accumulate at the leaf surface in secreting glandular trichomes (Smaoui et al. 2011). The influx of Na^+ into salt bladders of *Atriplex* species requires specific HKT1;2 transporter (Guo et al. 2023). Ben Hassine et al. (2009) demonstrated that ABA improved Na^+ excretion to bladder cells through an increase in polyamine synthesis in a coastal salt-tolerant Tunisian population of *A. halimus* but not in an inland population originating from a non-saline area. It is interesting to notice that the leaf ABA concentration in salt-treated plants was significantly higher for Djelfa than for Tam (Fig. 2A). The fact that Na^+ accumulation in Djelfa did not induce significant decrease in K^+ content also supports the halophytic nature of this population: the maintenance of K^+ concentration was often considered as an important component of salinity resistance since Na^+ may not substitute to K^+ for important metabolic functions (Pan et al. 2016; Rahman et al. 2021; Atta et al. 2023; Fu and Yang 2023; Mann et al. 2023).

Although salt stress induces an osmotic constraint on plants, response to salinity and to nonionic osmotic stress may strongly differ and this underlines the importance of ionic toxicity in salt-induced symptoms. Nemat-Alla et al. (2012) used a metabolomic approach on salt- and PEG-treated *A. halimus* and demonstrated that 87% of identified metabolites involved were unique to NaCl and 46% were unique to PEG. Similarly, Khedr et al. (2011) provided evidence that a DREB gene from *A. halimus* was induced by osmotic but not by ionic stress while Ghanmi et al. (2023) recently demonstrated that a FSK_2-type dehydrin in *A. halimus* was quite more expressed in response to water stress than in response to salinity.

Sodium was present only at low concentrations in PEG-containing solutions: however, water stress tolerant plants from Tam population significantly increased their Na^+ concentrations in all organs while such an increase was not recorded in Djelfa. This confirms that Na^+ assumes key (but still unidentified) functions in water stress resistance of *A. halimus* as previously demonstrated by Martínez et al. (2005). The PEG-induced increase in Na^+ was too low to contribute to osmotic adjustment. Qiu et al. (2003) postulated that Na^+ may increase carotenoid concentration in *A. centralasiatica* affording protection to photosynthetic apparatus. Although the total carotenoid concentration increased in Djelfa exposed to NaCl (Fig. 2D), it did not significantly increase in Tam exposed to PEG. In response to PEG, K^+ concentration also increased in roots and shoots of Tam, but not in Djelfa (Table 2) and this might be regarded as an attempt to reduce Ψs value which were significantly lower in Tam than in Djelfa for PEG-treated plants.

Different Osmocompatible Solutes are Involved in Salt and Osmotic Stress Tolerance

The present work suggests that the nature of the external constraint influences the nature of accumulated organic solutes. The salt-tolerant Djelfa indeed always accumulated higher concentrations of GB than Tam and its leaf GB concentration was higher in response to NaCl than in response to PEG. In contrast, the water stress-resistant Tam was almost unable to accumulate GB but accumulated proline, and to a higher extent in response to PEG than in response to NaCl. Beside its role in osmotic adjustment, proline is involved in oxidative stress management, protection of cellular structures and cytosolic pH buffering (Verbruggen and Hermans 2008; Lutts et al. 1999; Zheng et al. 2021; Zulfiqar and Ashraf 2023; Alvarez et al. 2022; Raza et al. 2023). Proline may also be used as a precursor for the synthesis of cell wall hydroxyproline involved in cell wall hardening. Benzarti et al. (2014) demonstrated that a more rigid cell wall allows the maintenance of cell/tissue integrity and contribute to maintenance of low water potential in *A. portulacoides*. An increase in P5C (Table 3) which is the immediate precursor of proline was observed in Tam but not in Djelfa and reflected a stimulation of the cytosolic glutamate pathway (La et al. 2020) in response to water stress in the former but not in the latter. Even if proline accumulated to higher concentration in response to PEG than to NaCl, Tam always contained higher concentration of proline, even in response to salinity, confirming that the capacity of proline over synthesis was clearly a property of Tam population.

GB also assumes several protective functions in plants (Annunziata et al. 2019; Ali et al. 2020b; Chen and Murata 2008; Huang et al. 2020). Choline is a precursor of GB: while it increased in response to stress in Djelfa (Table 3), it remained constant in Tam. Nuccio et al. (2000) reported that the choline transfer from cytosol into chloroplasts limits GB synthesis but in the case of Tam, the inability to synthesize the precursor appeared a more limiting factor than its chloroplastic translocation. According to Tabuchi et al. (2005), the choline precursor phosphocholine is produced by successive D-adenosyl-L-methionine (*S*-adenosyl-methionine (SAM))-dependent *N* methylation of phosphoethanolamine. Ben Hassine and Lutts (2010)

Table 4 Leaves and roots betaine aldehyde dehydrogenase activity (BADH) in seedlings of *Atriplex halimus* L. originating from the salt-affected area (Djelfa) or the arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1})

Treatment	Population	BADH activity (nmol NAD^+ mg^{-1} prot min^{-1})	
		Leaves	Roots
Control	Djelfa	111 ± 9 a	94 ± 5 a
	Tam	101 ± 8 a	112 ± 11 a
Mild stress			
NaCl 100 mM	Djelfa	353 ± 22 c	208 ± 17 c
	Tam	123 ± 10 a	125 ± 13 ab
PEG 50 g L^{-1}	Djelfa	227 ± 15 b	223 ± 21 c
	Tam	114 ± 11 a	138 ± 18 b
Severe stress			
NaCl 300 mM	Djelfa	622 ± 45 d	501 ± 38 e
	Tam	213 ± 14 b	98 ± 11 a
PEG 100 g L^{-1}	Djelfa	388 ± 32 c	411 ± 29 d
	Tam	251 ± 31 b	105 ± 9 a

Each value is the mean of four replicates ± standard errors. For a given parameter and organ, means with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test

ND not detected

mentioned that in the presence of PEG, some accessions of *Atriplex halimus* produce very high amounts of ethylene. Since ethylene is also produced from SAM, oversynthesis of ethylene in stressed plants from Tam could induce SAM depletion which hinder choline production. Quantification of ethylene synthesis by Tam should help us to test this hypothesis.

Although GB was reported to accumulate in plants exposed to drought or chilling stress (Qin et al. 2017; Huang et al. 2020; Wang et al. 2019), Subbarao et al. (2001) clearly demonstrated that Na^+ increase is required to stimulate GB synthesis. Egan et al. (2001) compared the effects of various salts on GB synthesis by *Atriplex prostrata* and concluded that only Na^+ salts trigger synthesis of the quaternary ammonium compound. In *Atriplex gmelini*, Tsutsumi et al. (2015) detected high concentration of GB in bladder cells exhibiting a high amount of Na^+, reinforcing the hypothesis of a specific role of GB in response to Na^+ constraint.

Proline and GB should not be regarded as mutually exclusive. Wang et al. (2019) even reported that GB accumulation may increase proline production in *Atriplex hortensis* and Qin et al. (2017) reported a similar observation in *Atriplex canescens*. One of the main differences between proline and GB is related to the fact that proline is quickly recycled after the stress relief while GB is hardly metabolized. Tam originated from an inland area with low episodic rainfall: in these conditions, proline may be rapidly recycled after rainfall to sustain growth during the recovery period (Nada et al. 2022). In contrast, Djelfa originated from a highly saline area where salt is permanently present and this may explain that GB which is hardly catabolized in plant tissues is a suitable protective compound to thrive in these harsh conditions.

Total soluble sugars increased in response to NaCl and PEG. Martínez et al. (2005) considered that TSS were the major contributors to osmotic adjustment in *Atriplex halimus* exposed to PEG. Verslues and Bray (2006) consider in the glycophyte *Arabidopis thaliana* that proline and sugar accumulation may be connected since sugars may stimulate proline synthesis and that such interaction is modulated by ABA. In the present work, TSS (Fig. 3E) and ABA (Fig. 2A) increased in leaves of Djelfa exposed to NaCl while proline remained rather low, suggesting that this model is not valid, or is more complex, in the halophyte *A. halimus*.

Enzymatic Regulation of Osmotic Adjustment

The present work suggests that not only the nature of accumulated solute but also the modalities of enzymatic regulation of the concerned compound vary depending on the nature of the constraint and the identity of population. The current study indeed showed that salt-induced proline accumulation in Tam was related to higher activity of P5CR (Fig. 4A) while proline accumulation in response to PEG was also due to an increase in OAT activity (Fig. 4). In contrast, proline accumulation in Djelfa may have occurred as a result of PDH inhibition.

P5CS activity was not quantified in the present work due to the lack of available methods allowing to accurately measured this activity. According to Verbruggen and Hermans (2008), P5CS activity represents a rate-limiting step of proline synthesis. The level of P5C recorded in Djelfa comparatively to Tam suggests that this may be the case in response to PEG but not in response to NaCl (Table 3). However, the higher P5CR activity recorded in salt-treated Tam than in Djelfa may also suggests that P5C produced by P5CS in Tam was rapidly converted to proline thus partly explaining the higher proline content in Tam than in Djelfa. According to La et al. (2020), a glutamate increase in plant tissue may trigger proline accumulation through P5CS-P5CR pathway. Lutts et al. (1999) showed that proline accumulation in rice may be related to an increase in the endogenous pool of glutamate and an increase in glutamate dehydrogenase deaminating activity.

P5CR in plants is present in chloroplasts and in cytosol (Raza et al. 2023). In response to osmotic stress, availability of CO_2 may be reduced as a consequence of stomatal closure. NADPH consumption by the Calvin cycle is then decreased and proline synthesis in chloroplasts may recycle $NADP^+$ produced by the

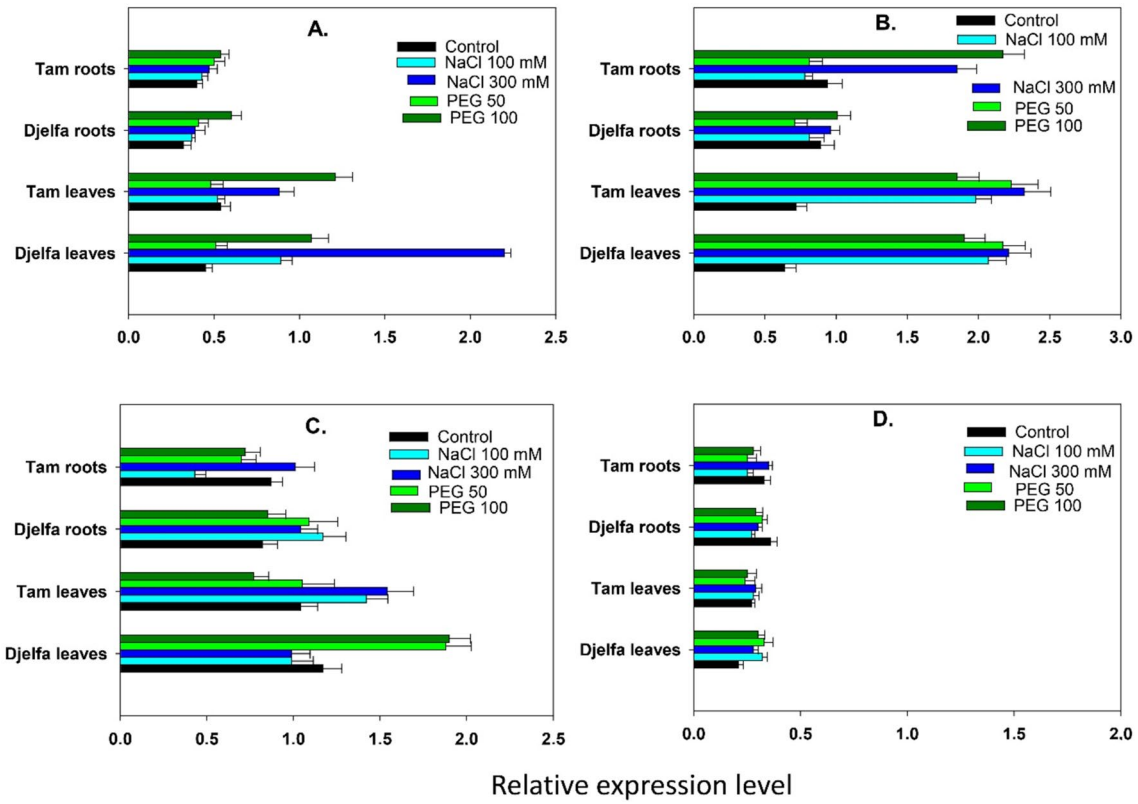


Fig. 5 Semi-quantitative RT-PCR expression analysis of *P5CS1* (**A**), *P5CR* (**B**), *PDH* (**C**) and *OAT* (**D**) in leaves and roots of *Atriplex halimus* originating from the salt-affected area (Djelfa) or from the non-saline arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1}). Each value is the mean of four replicates ± standard errors. Bars with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test

electron transport chain, thus avoiding ROS production (Alvarez et al. 2022; Zulfiqar and Ashraf 2023). When data were pooled for roots and leaves for the two considered populations, a positive correlation was found between P5CR activities and proline concentration ($r^2 = 0.79$ ** for Djelfa and $r^2 = 0.70$ * form Tam) (Fig. 7A). Similarly, a positive correlation was found for both populations between OAT activities and proline concentration (Fig. 7B). OAT activity was increased in Tam, mainly in the presence of PEG. OAT is located in the mitochondria where it is involved in transamination of ornithine to P5C (Zheng et al. 2021). Proline in mitochondria may serve as a sink for soluble nitrogen. Ornithine is also a precursor of polyamines which are key regulatory molecules in stressed plants; an increase in ornithine availability may result from a decrease in polyamine synthesis resulting from the consumption of SAM for choline synthesis. However, the recorded increase in OAT activity was recorded only for Tam which produced a low amount of choline. PDH is also located in the mitochondria and is frequently inhibited during stress and activated during recovery (Verbruggen and Hermans 2008; Zheng et al. 2021). In the present work, we showed that stress-induced inhibition strongly differed between the two populations but no correlation between PDH activity and proline accumulation was found in the present study (Fig. 7C).

Choline monooxygenase is considered as the rate-limiting step for GB synthesis (Kurepin et al. 2015; Mansour and Ali 2017; Chen and Murata 2008). CMO activity could not be measured in enzyme extracts by classical methods but our work showed that Djelfa and Tam strongly differed for choline concentrations (Table 3) leading us to hypothesize that the availability of choline was also a limiting factor hampering GB synthesis in Tam. Moreover, the recorded BADH activity involved in the second step of GB synthesis remained rather low in Tam under all stress conditions. A significant correlation between BADH activity and GB concentration was noticed for both population ($r^2 = 0.72$ ** for Djelfa; $r^2 = 0.64$ * for Tam) (Fig. 7D) suggesting that BADH activity may have a strong impact on the regulation of GB concentration.

Both proline and GB may be transported in the plant over long distance. ProT belongs to a family of plasma membrane-located transporter able to mediate the transport of both compounds between organs. According to Grallath et al. (2005), ProT is even more efficient for

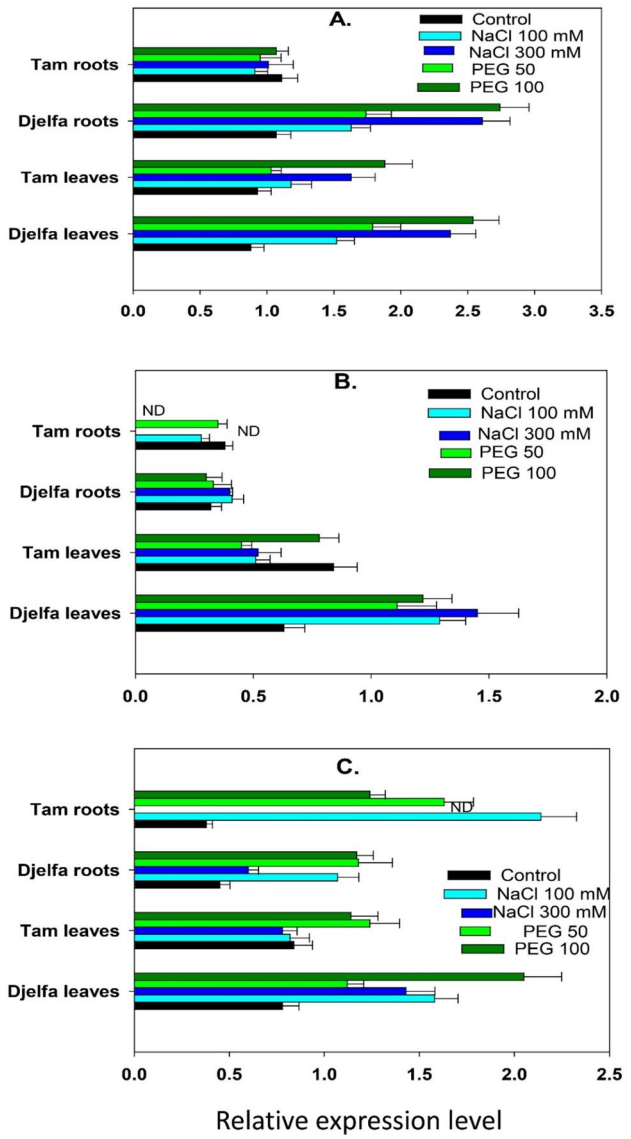


Fig. 6 Semi-quantitative RT-PCR expression analysis of *ProT* (**A**), *CMO* (**B**) and *BADH* (**C**) in leaves and roots of *Atriplex halimus* originating from the salt-affected area (Djelfa) or from the non-saline arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1}). Each value is the mean of four replicates ± standard errors. Bars with similar letters were not different at $P < 0.05$ according to the Tukey's HSD test

GB than for proline transport. Gene coding for ProT was upregulated (Fig. 6A), especially in Djelfa, and to a higher extent in response to NaCl than in response to PEG. In *Arabidopsis thaliana*, gene expression was found in the phloem parenchyma throughout the whole plant indicative of a long-distance transport. Considering that GB is mainly produced in chloroplasts, it may be hypothesized that ProT contributes to GB transfer from the shoot to the roots, at least in Djelfa, although detection of measurable BADH activity in the root also suggests that a root synthesis of GB may occur in these plants.

Stress-Induced Modifications of Enzyme Activities are Hardly Explained by Transcriptional Regulation

Although we found a significant correlation between enzyme activities and compatible solutes (Fig. 7), we did not find such a strong correlation between enzyme activities and the abundance of the corresponding transcripts. Since roots and leaves are supposed to display contrasting regulatory mechanisms in Atriplex species (Silveira et al. 2009), we analyzed the relationships between the relative modification in transcript abundance (RM_{TA}) and the relative modification in enzyme activity (RM_{EA}) separately for the two types of organs (Fig. 8). A low ($r^2 = 0.61$*) correlation was found between RM_{TA} and RM_{EA} for root P5CR while an unexpected negative correlation ($r^2 = 0.67$*) was found for the leaves. No significant correlation was found between RM_{TA} and RM_{EA} for the other enzymes. This implies that in *Atriplex halimus*, synthesis of osmocompatible solutes is poorly transcriptionally regulated. Numerous studies established a direct link between final product accumulation (proline or GB) and gene expression without considering enzyme activities (Verbruggen and Hermans 2008; Bai et al. 2022): hence, one "piece of the puzzle" is missing. The absence of significant correlation between RM_{TA} and RM_{EA} does not imply that the constitutive expression of the gene was sufficient to ensure osmocompatible solute synthesis in stress conditions. Indeed, RM_{TA} values were always significantly different from 1: most genes except the gene coding for OAT were significantly up- (*P5CR*, *P5CS*, *BADH*, *CMO*) or down (*PDH*) regulated. Our data however indicate that post-transcriptional regulatory processes are of crucial importance for the regulation of enzyme activities involved in proline and GB synthesis in *A. halimus*.

Numerous data mentioning transcriptional regulation of key enzymes come from heterologous expression of a transgene, very often under the control of 35S promoter (Jia et al. 2002; Fu et al. 2011; Di et al. 2015; Wang et al. 2019; Ali et al. 2020b) but the situation *in planta* appears quite more complex. Hua et al. (2001; cited by Verbruggen and Hermans 2008) reported that *AtP5CR* transcripts accumulated in response to heat stress without further protein level and that 5′-UTR leader sequence of *AtP5CR* mRNA is involved in stabilization and translation inhibition. In a wider study, Kubala et al. (2015) studied 952 genes involved in seed priming using an *omic* approach and showed that transcription was not coordinately associated with translation resulting in a limited correspondence between mRNA levels and protein abundance. In the present work, in vitro measurements of enzyme activities were performed under optimal conditions with adequate

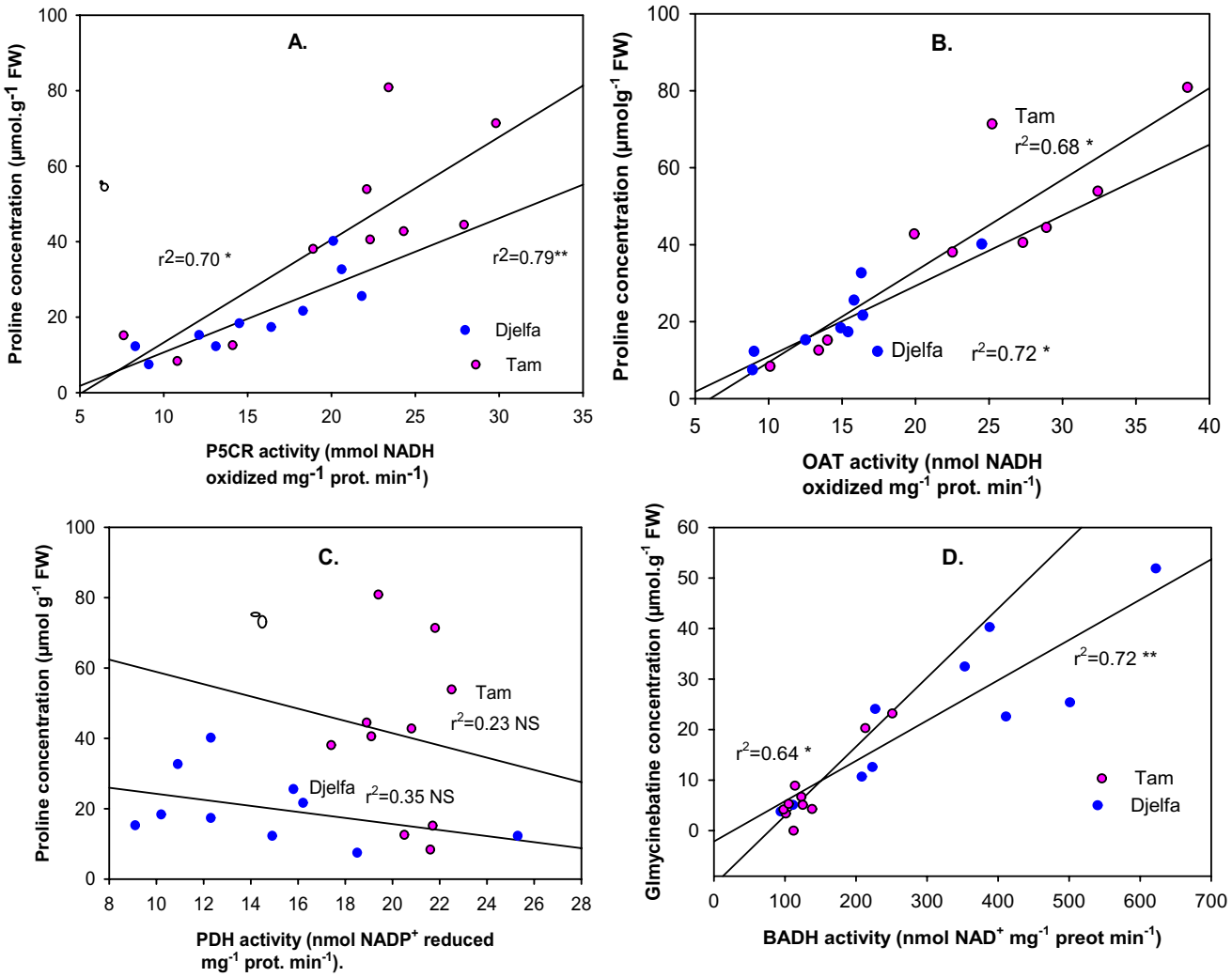


Fig. 7 Correlation between enzyme activities and osmocompatible solute concentration: Δ^1-pyrroline-5-carboxylate reductase (P5CR) and proline concentration (**A**) proline dehydrogenase (PDH) and proline concentration (**B**) ornithine δ-aminotransferase (OAT) and proline concentration (**C**) betaine aldehyde dehydrogenase (BADH) and glycinebetaine concentrations. Data were pooled for leaves and roots of *Atriplex halimus* originating from the salt-affected area (Djelfa) or from the non-saline arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1}). Each point is the mean of eight replicates betaine aldehyde dehydrogenase (BADH) and glycinebetaine concentrations (**D**)

supply of co-factors and substrate and this may not fully reflect the actual enzyme activity in vivo within tissues. Nevertheless, inferring metabolism regulation on the basis of gene expression without considering enzyme activities may be regarded as an oversimplification because it neglects the crucial process of post-transcriptional regulation.

Proline and GB may accumulate through both an ABA-dependent and ABA-independent pathways. In *Atriplex halimus*, ABA accumulated at the leaf level in response to all stress treatments (Fig. 2A). An increase in ABA may contribute to proline synthesis and abscisic acid-responsive elements were reported in promoter of numerous genes (Verbruggen and Hermans 2008; La et al. 2020). Guo et al. (2016) reported that *GmProT1* coding for proline/GB transporter in soybean is expressed in response to ABA treatments. Sharma and Verslues (2010) however provided clear evidences that P5CS, PDH and OAT may also regulate

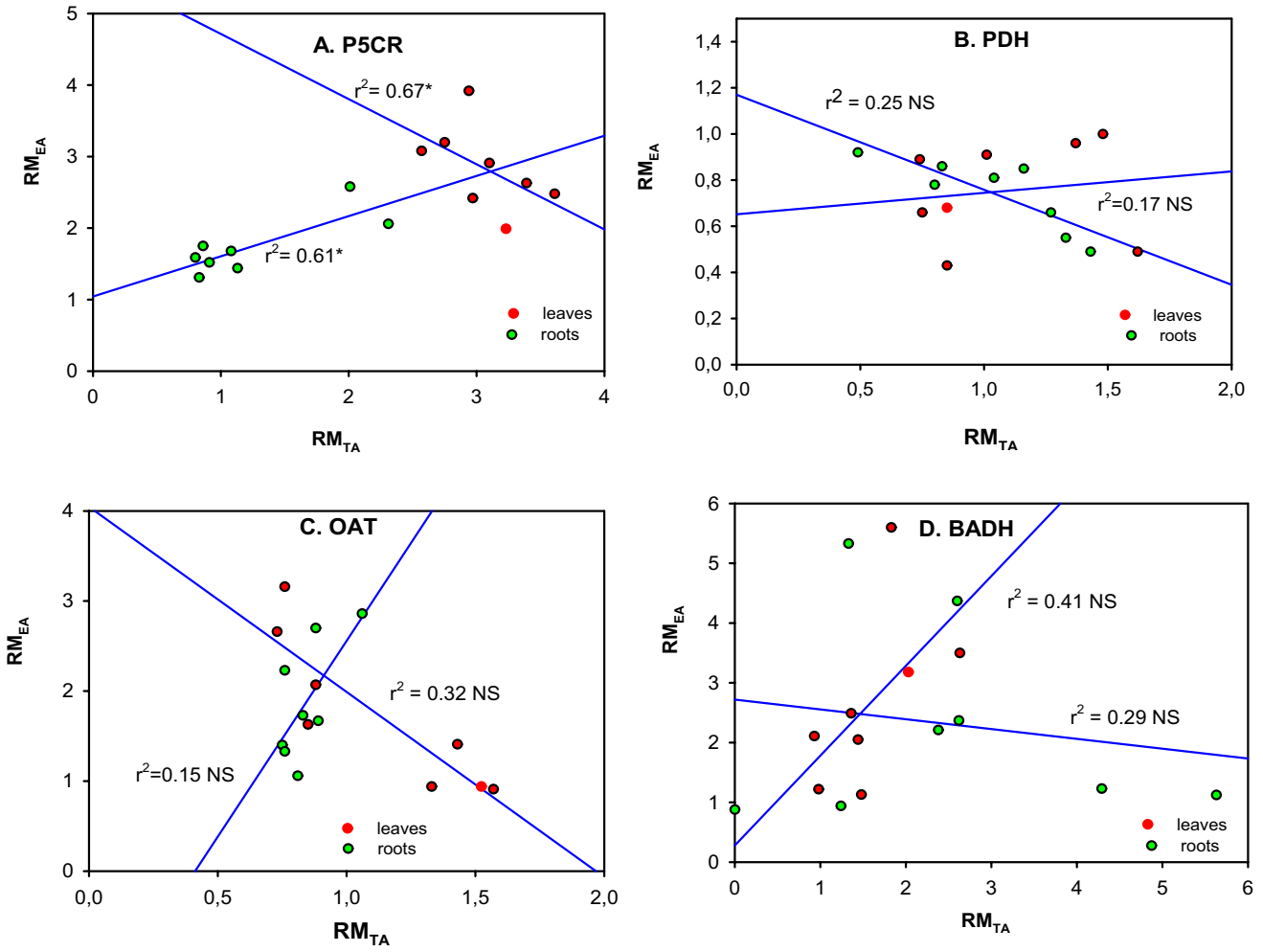


Fig. 8 Relationships between the relative modification of transcript abundance (RM_{TA}) and the relative modification of enzyme activities (RM_{EA}) in leaves (red points) and roots (green points) for Δ^1-pyrroline-5-carboxylate reductase (**A**), proline dehydrogenase (**B**), ornithine δ-aminotransferase (**C**) and betaine aldehyde dehydrogenase (**D**). Data were pooled for two populations of *Atriplex halimus* originating from the salt-affected area (Djelfa) or the arid area (Tamanrasset (Tam)) and exposed during 10 days to mild stress (NaCl 100 mM or PEG 50 g L^{-1}) or to severe stress (NaCl 300 mM or PEG 100 g L^{-1}). Each point is the mean of eight replicates (Color figure online)

proline status independently of ABA. Numerous studies reported that ABA may trigger the expression of genes coding for CMO or BADH (Kurepin et al. 2015; Mansour and Ali 2017; Annunziata et al. 2019) but Wang and Showalter (2004) demonstrated that this is not the case in the halophyte *Atriplex prostrata*. We may not exclude that modalities of gene regulation are not the same for glycophyte and halophyte plant species (Khedr et al. 2011) and may differ between plant tissues, or even leaves of different ages during development (Nada and Abogadallah 2015; Nada et al. 2018) in relation to organ sensitivity to ABA (Verslues and Bray 2006).

Conclusion

Two populations of the xero-halophyte species *Atriplex halimus* exhibited contrasting behavior in terms of osmocompatible solute accumulation depending on their habitat of origin. The population issued from a salt-affected zone (Djelfa) was more resistant to salinity than the population originating from a non-saline xeric habitat (Tamarasset) whereas the opposite was true for resistance to nonionic water stress induced by PEG. Although both populations accumulated proline and glycinebetaine for osmotic adjustment, the salt-resistant population Djelfa mainly accumulated glycinebetaine while the drought-resistant population Tamanrasset mainly accumulated proline. Salt-induced proline accumulation was related to

higher activity of Δ^1-pyrroline-5-carboxylate reductase (EC 1.5.1.2) while proline accumulation in response to PEG was also due to an increase in ornithine δ-aminotransferase (EC 2.6.1.13) activity. Glycinebetaine accumulation was related to a high chloroplastic betaine aldehyde dehydrogenase activity (EC 1.2.1.8) while a low synthesis of the precursor choline may also compromise glycinebetaine synthesis. Although a significant correlation was found between enzyme activities and compatible solutes concentrations, no correlation was detected between enzyme activities and the corresponding gene expression. Post-transcriptional regulation processes may thus assume key functions in proline and glycinebetaine synthesis in *Atriplex halimus*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-024-11558-7.

Acknowledgements The authors are grateful to the MOCA analytical platform of UCLouvain and to Mrs. Hélène Dailly for their precious help in analytical procedures. This work is dedicated to the memories of Dr Selma Kebbas (University of Blida, Algeria) and Dr. Gilles Guerrier (University of Orléans; France).

Author Contributions LC performed the experimental work and treatment of the data; CC managed the project and contributes to assessment of enzyme activities; JPM contributed to statistical treatment of the data, interpretation of obtained results and contributes to assessment of enzyme activities; MQ performed molecular analysis in relation to gene expression; SL managed the project and wrote the first draft of the manuscript; All authors contributed and approved the final version of this manuscript.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

References

Ali S, Abbas Z, Seleiman MF, Rizwan M, Yavas I, Alhammad BA, Shami A, Hasanuzzaman M, Kalderis D (2020a) Glycine betaine accumulation, significance and interests for heavy metal tolerance in plants. Plants 9:896

Ali A, Ali Q, Iqbal MS, Nasir IA, Wang X (2020b) Salt tolerance of potato genetically engineered with the *Atriplex canescens BADH* gene. Biol Plant 64:271–279

Alotibi MM, Al-Huqail AA, Ghoneim AM, Eissa MA (2023) Seasonal variations in yield and biochemical composition of the Mediterranean salt bush (*Atriplex halimus*) under saline agriculture in semi-arid régions. J Soil Sci Plant Nutr 23:3834–3844

Alvarez ME, Savouré A, Szabados L (2022) Proline metabolism as regulatory hub. Trends Plant Sci 27:39–55

Annunziata MFG, Ciarmiello LF, Woodrow P, Dell'Aversana E, Carillo P (2019) Spatial and temporal profile of glycine betaine accumulation in plants under abiotic stresses. Front Plant Sci 10:230

Atta K, Mondal S, Gorai S, Singh AP, Kumari A, Ghosh T, Roy A, Hembram S, Gaikwad DJ, Mondal S, Bhattacharya S, Jha UC, Jespersen D (2023) Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. Front Plant Sci 14:1241736

Bai M, Zeng W, Chen F, Ji X, Zhung Z, Jin B, Wang J, Jia L, Peng Y (2022) Transcriptome expression profiles reveal response mechanisms to drought and drought-stress mitigation mechanisms by exogenous glycine betaine in maize. Biotechnol Lett 44:367–386

Bajji M, Lutts S, Kinet JM (2000) Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus cultures issued from durum wheat (*Triticum durum* Desf.) cultivars differing in drought resistance. J Plant Physiol 156:75–83

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Belkheiri O, Mulas M (2013) The effects of salt stress on growth, water relations and ion accumulation in two halophyte *Atriplex* species. Environ Exp Bot 86:17–28

Ben Hassine A, Lutts S (2010) Differential responses of saltbush *Atriplex halimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene. J Plant Physiol 167:1448–1456

Ben Hassine A, Ghanem ME, Bouzid S, Lutts S (2008) An inland and a coastal population of the Mediterranean xero-halopte species *Atriplex halimus* L differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. J Exp Bot 59:1315–1326

Ben Hassine A, Ghanem ME, Bouzid S, Lutts S (2009) Abscisic acid has contrasting effects on salt excretion and polyamine concentrations of an inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus*. Ann Bot 104:9256–9936

Ben Hassine A, Bouzid S, Lutts S (2010) Does habitat of *Atriplex halimus* L. affect plant strategy for osmotic adjustment ? Acta Physiol Plant 32:325–331

Benzarti M, Ben Rejeb K, Messedi D, Ben Mna A, Hessini K, Ksontini M, Abdelly C, Debez A (2014) Effect of high salinity in *Atriplex portulacoides*: growth, leaf water relations and solute accumulation in relation with osmotic adjustment. S Afr J Bot 95:70–77

Bouchenak F, Henri P, Benrediha FZ, Rey P (2012) Differential responses to salinity of two *Atriplex halimus* populations in relation to organic solutes and antioxidant systems involving thiol reductases. J Plant Physiol 169:1445–1453

Bradford MM (1976) A rapid sensitive method for the quantification of microquantities of protein utilizing the principle of protein dye binding. Ana Biochem 161:559–566

Casasni L (2021) Caractérisation Ecophysiologique et Biochimique d'*Atriplex halimus* en Réponse aux Stress Abiotiques. PhD Thesis, Faculté des Sciences de la Nature et de la Vie, University of Blida, Algeria

Chen THH, Murata N (2008) Glycinebetaine: an effective protectant against abiotic stress in plants. Trends Plant Sci 13:9

Chen J, Wu J, Lu Y, Cao Y, Zeng Y, Zhang Z, Zhang L, Wang S (2016) Molecular cloning and characterization of a gene encoding the proline transporter protein in common bean (*Phaseolus vulgaris* L.). Crop J 4:384–390

Chen X, Zhao C, Yu M, Zhou M, Chen ZH, Shabala S (2023) Climate-resilient crops: lessons from xerophytes. Plant J 117:1815–1835

Di H, Tian Y, Zu H, Meng X, Zeng X, Wang Z (2015) Enhanced salinity tolerance in transgenic maize plants expressing a *BADH* gene from *Atriplex micrantha*. Euphytica 206:775–783

Dietz KJ, Zörb C, Geilfus CM (2021) Drought and crop yield. Plant Biol 23:881–893

Egan TP, Dewald HD, Ungar IA (2001) The effect of different salts of sodium and potassium on the accumulation of glycinebetaine in *Atriplex prostrata*. Biol Plant 44:595–597

Elbouzidi A, Ouassou H, Aherkou M, Kharchoufa L, Mzaskali N, Baraich A, Mechchate H, Bouhtim M, Idir A, Hano C, Zrouri H, Addi M (2022) LC-MS/MS phytochemical profiling, antioxidant activity, and cytotoxicity of the ethanolic extract of *Atriplex halimus* L. against breast cancer cell lines: computational studies and experimental validation. Pharmaceuticals 1:1156

Ennoury A, Roussi Z, Nhhala N, Zouaoui Z, Kabach I, Krid A, Kchikich A, Nhiri M (2023) *Atriplex halimus* water extract: a biochemical composition that enhanced the faba bean plants growth. Physiol Mol Biol Plants 29:601–611

Fu H, Yang Y (2023) How plant tolerate salt stress. Curr Issues Mol Biol 45:5914–5934

Fu XZ, Khan EU, Hu SS, Fan QJ, Liu JH (2011) Overexpression of the betaine aldehyde dehydrogenase gene from *Atriplex hortensis* enhances salt tolerance in the transgenic trifoliate orange (*Poncirus trifoliata* L. Raf.). Environ Exp Bot 74:106–113

Ghanmi S, Smith MA, Zaisi I, Drira M, Graeher SP, Hann M (2023) Isolation and molecular characterization of an FSK_2-dehydrin from *Atriplex halimus*. Phytochemistry 213:113783

Ghosh UK, Islam MN, Siddiqui MN, Cao X, Khan MAR (2022) Proline, a multifaceted signalling molecule in plant response to abiotic stress: understanding the physiological mechanisms. Plant Biol 24:227–239

Grallath S, Weimar T, Meyer A, Gumy C, Suter-Grotmeyer M, Neuhaus JM, Rentsch D (2005) The AtProT family. compatible solute transporters with similar substrate specificity but differential expression patterns. Plant Physiol 137:117–126

Grieve CM, Grattan SR (1983) Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70:303–307

Guo N, Xue D, Zhang W, Zhao JM, Xue CC, Yan Q, Xue JY, Wang HT, Zhang YM, Xing H (2016) Overexpression of *GmProT1* and *GmProT2* increases tolerance to drought and salt stresses in transgenic *Arabidopsis*. Sci Direct 15:1727–1743

Guo H, Cui YN, Zhang L, Feng S, Ren ZJ, Wang SM, Bao AK (2023) *AcHKT1;2* is a candidate transporter mediating the influx of Na^+ into the salt bladder of *Atriplex canescens*. Plant Soil 483:607–604

Haddioui A, Baaziz M (2001) Genetic diversity of natural populations of *Atriplex halimus* L. in Morocco: an isoenzyme-based overview. Euphytica 121:99–106

Haile GG, Tang Q, Li W, Liu X, Zhang X (2020) Drought: progress in boardening its understanding. WIREs Water 7:e1407

Hamrouni L, Hanana M, Abdely C, Ghorbel C (2011) Exclusion du chlorure et inclusion du sodium : deux mécanismes concomitants de tolérance à la salinité chez la vigne sauvage *Vitis vinifera* subsp. *sylvestris* (var Séjène). Biotechnol Agron Soc Environ 15:387–400

Huang S, Zuo T, Ni W (2020) Important roles of glycinebetaine in stabilizing the structure and function of the photosystem II complex under abiotic stresses. Planta 251:36

Jia GX, Zhu ZQ, Chang FQ, Li YX (2002) Transformation of tomato with the *BADH* gene from *Atriplex* improves salt tolerance. Plant Cell Rep 21:141–146

Khedr AHA, Serag MS, Nemat-Alla MM, Abo-Elnaga AZ, Nada RM, Quick WP, Abogadallah GM (2011) A *DREB* gene from the xero-halophyte *Atriplex halimus* is induced by osmotic but not ionic stress and shows distinct differences from glycophytic homologues. Plant Cell Tissue Organ Cult 106:191–206

Khostavichemar A, Aalijahan M, Moaazeni S, Lupo AR, Karimi A, Ulrich M, Parvian N, Sadeghi A, von Suchodoletz H (2023) Assessing a multi-method approach for dryland soil salinization with respect to climate change and global warming—the example of the Bajestan region (NE Iran). Ecol Ind 154:110639

Kubala S, Garnczarska M, Wojtyla L, Clippe A, Kosmala A, Żmieńko A, Lutts S, Quinet M (2015) Deciphering priming-induced improvement of rapeseed (*Brassica napus* L.) germination through an integrated transcriptomic and proteomic approach. Plant Sci 231:94–113

Kurepin LV, Ivanov AG, Zaman M, Pharis RP, Allakherdiev SI, Hurry V, Hüner NPA (2015) Stress-related hormones and glycinebetaine interplay in protection of photosynthesis under abiotic stress conditions. Photosynth Res 126:221–235

La VH, Lee BR, Islam MT, Mamun MA, Park SH, Bae DW, Kim TH (2020) Characterization of glutamate-mediated hormonal regulatory pathway of the drought responses in relation to proline metabolism in *Brassica napus* L. Plants 9:512

Le Houérou HH (2000) Utilization of fodder trees and shrubs in the arid and semiarid zones of West Asia and North Africa. Arid Soil Res Rehabil 14:101–135

Lichtenthaler HK (1987) Chlorophylles and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382

Litalien A, Zeeb B (2020) Curing the earth: a review of anthropogenic soil salinization and plant-based strategy for sustainable mitigation. Sci Total Environ 698:134235

Lutts S, Majerus V, Kinet JM (1999) NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. Physiol Plant 105:450–458

Lutts S, Lefèvre I, Delpérée S, Kivits S, Deschamps C, Robledo A, Correal E (2004) Heavy metal accumulation by the halophyte species Mediterranean saltbush. J Environ Qual 33:1271–1279

Mann A, Lata C, Kumar N, Kumar A, Kumar A, Sheoran P (2023) Halophytes as new model plant species for salt tolerance strategies. Front Plant Sci 14:1137211

Mansour MMF, Ali EF (2017) Glycinebetaine in saline conditions: an assessment of the current state of knowledge. Acta Physiol Plant 39:56

Martínez JP, Kinet JM, Bajji M, Kutts S (2005) NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus*. J Exp Bot 56:2421–2431

Mezl VA, Knox WE (1976) Properties and analysis of a stable derivative of pyrroline-5-carboxylic acid for use in metabolic studies. Anal Biochem 74:430–440

Nada RM, Abogadallah GM (2015) Developmental acquisition of salt tolerance in the halophyte *Atriplex halimus* L. is related to differential regulation of salt inducible genes. Plant Growth Regul 75:165–178

Nada RM, Khedr AHA, Serag MS, El-Qashlam NR, Abogadallah GM (2018) Maturation of *Atriplex halimus* L. leaves involves changes in the molecular regulation of stomatal conductance under high evaporative demand and high but not low soil water content. Planta 248:795–812

Nada RM, Khedr AHA, Serag MS, El-Qashlam NR, Abogadallah GM (2022) Molecular and physiological responses of naturally grown *Atriplex halimus* L. to drought-stress recovery in the absence or presence of Na^+ ions under natural conditions. J Plant Growth Regul 41:1578–1593

Nemat-Alla MM, Khedr AHA, Serag MM, Abu-Almaga AZ, Nada RM (2011) Physiological aspects of tolerance in *Atriplex halimus* L. to NaCl and drought. Acta Physiol Plant 33:547–557

Nemat-Alla MM, KhedrAHA SMM, Abu-Almaga AZ, Nada RM (2012) Regulation of metabolomics in *Atriplex halimus* growth under salt and drought stress. Plant Growth Regul 67:281–304

Nuccio ML, McNeil SD, Ziemak MJ, Hanson AD (2000) Choline import to chloroplasts limits glycine betaine synthesis in tobacco: analysis of plants engineered with a chloroplastic or a cytosolic pathway. Metabol Eng 2:300–311

Ortíz-Dorda J, Martínez-Mora C, Correal E, Simón B, Cenis JL (2005) Genetic structure of *Atriplex halimus* populations in the Mediterranean basin. Ann Bot 95:827–834

Otal J, Orengo J, Quiles A, Hevia ML, Fuentes F (2010) Characterization of edible biomass of *Atriplex halimus* L. and its effect on feed and water intakes, and on blood mineral profile in non-pregnant Manchega-breed sheep. Small Rum Res 91:208–214

Pan YQ, Guo Y, Wang SM, Zhao B, Zhang JL, Ma Q, Yin HJ, Bao AK (2016) The photosynthesis, Na^+/K^+ homeostasis and osmotic adjustment of *Atriplex canescens* in response to salinity. Front Plant Sci 7:848

Pérez-Romero JA, Mateos-Naranjo E, López-Jurado J, Redondo-Gómez S, Torres-Ruiz JM (2020) Importance of physiological traits vulnerability in determine halophytes tolerance to salinity excess; a comparative assessment in *Atriplex halimus*. Plants 9:690

Qin D, Zhao CL, Liu XY, Wang PW (2017) Transgenic soybeans expressing betaine aldehyde dehydrogenase from *Atriplex canescens* show increased drought tolerance. Plant Breed 136:699–709

Qiu N, Lu Q, Lu C (2003) Photosynthesis, photosystem II efficiency and the xantophyll cycle in salt-adapted halophyte *Atriplex centralasiatica*. New Phytol 159:479–486

Quinet M, Bataille G, Dobrev PI, Capel C, Gómez P, Capel J, Lutts S, Motyka V, Angosto T, Lozano R (2014) Transcriptional and hormonal regulation of petal and stamen development by *STAMENLESS*, the tomato (*Solanum lycopersicum* L.) orthologue to the B-class *APETALA* gene. J Exp Bot 65:2243–2256

R Development Core Team (2009). R: A language environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing

Rahman MM, Mostofa MG, Keya SS, Siddiqui MN, Ansary MMU, Das AK, Rahlman MA, Tran LSP (2021) Adaptative mechanisms of halophytes and their potential in improving salinity tolerance in plants. Int J Mol Sci 22:10733

Raza A, Charagh S, Abbas S, Hassan MU, Saeed F, Haider S, Sharif R, Anand A, Corpas FJ, Kin W, Varshney RK (2023) Assessment of proline function in higher plants under extreme temperature. Plant Biol 25:379–395

Saneoka H, Ishiguro S, Moghaieb REA (2001) Effect of salinity and abscisic acid on accumulation of glycinebetaine and betaine aldehyde dehydrogenase mRNA in sorghum leaves (*Sorghum bicolor*). J Plant Physiol 158:853–859

Sharma S, Verslues PE (2010) Mechanisms independent of abscisic acid (ABA) or proline feedback have a predominant role in transcriptional regulation of proline metabolism during low water potential and stress recovery. Plant Cell Environ 33:1838–1851

Silveira JAG, Araújo SAM, Lima JPMS, Viégas RM (2009) Roots and leaves display contrasting adjustment mechanisms in response to NaCl-salinity in *Atriplex nummularia*. Environ Exp Bot 68:1–8

Smaoui A, Barhoumi Z, Rabhi M, Abdelly C (2011) Localization of potential ion transport pathways in vesicular trichome cells of *Atriplex halimus* L. Protoplasma 248:363–372

Song Y, Gao M, Xu Z, Wang J, Bi M (2023) Temporal and spatial characteristics of soil salinization and its impact on cultivated land productivity in the BOHAI Rim region. Water 15:2368

Spormann S, Nadais P, Sousa F, Pinto M, Martins M, Sousa B, Fidalgo F, Soares C (2023) Accumulation of proline in plants under contaminated soils. Are we on the same page? Antioxidant 12:66

Subbarao GV, Wheeler RM, Levine LH, Stutte GW (2001) Glycinebetaine accumulation, ionic and water elations of redbeet at contrasting levels of sodium supply. J Plant Physiol 158:767–776

Tabuchi T, Kawaguchi Y, Zuma T, NanmoriT YT (2005) Similar regulation patterns of choline monooxygenase, phosphoethanolamine *N*-methyltransferase and *S*-adenosyl-L-methionine synthetase in leaves of the halophyte *Atriplex nummularia*. Plant Cell Physiol 46:505–513

Tsutsumi K, Yamada N, Cha-um S, Tanaka Y, Takabe T (2015) Differential accumulation of glycinebetaine and choline monooxygenase in bladder hairs and lamina leaves of *Atriplex gmelini* under high salinity. J Plant Physiol 176:101–107

Verbruggen N, Hermans S (2008) Proline accumulation in plants: a review. Amino Acids 35:753–759

Verslues PE, Bray EA (2006) Role of abscisic acid (ABA) and *Arabidopsis thaliana* ABA-insensitive loci in low water potential-induced ABA and proline accumulation. J Exp Bot 57:201–212

Walker DJ, Moñino I, González E, Frayssinet N, Correal E (2005) Determination of ploidy and nuclear DNA content in populations of *Atriplex halimus* (Chenopodiaceae). Bot J Linn Soc 147:441–448

Walker DJ, Lutts S, Sánchez-García M, Correal E (2014) *Atriplex halimus* L.: its biology and uses. J Arid Environ 100/101:111–121

Wang LW, Showalter AM (2004) Cloning and salt-induced, ABA-independent expression of choline mono-oxygenase in *Atriplex prostrata*. Physiol Plant 120:405–412

Wang Y, Liang C, Meng Z, Li Y, Abid MA, Askari M, Wang P, Wang Y, Sun G, Cai Y, Chen SY, Lin Y, Zhang R, Guo S (2019) Leveraging *Atriplex hortensis* choline monooxygenase to improbe chilling tolerance in cotton. Environ Exp Bot 162:364–373

Weretilnyk EA, Hanson AD (1986) Betaine aldehyde dehydragenase from spinach leaves: purification, *in vitro* translation of the mRNA and regulation by salinity. Arch Biochem Biophys 271:56–63

Yemm EW, Willis J (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514

Zheng Y, Cabassa-Hourton C, Planchais S, Lebreton S, Savouré A (2021) The proline cycle as an eukaryotic redox valve. J Exp Bot 772:6856–6866

Zhou MX, Renard ME, Quinet M, Lutts S (2019) Effect of NaCl on proline and glycinebetaine metabolism in *Kosteletzkya pentacarpos* exposed to Cd and Zn toxicities. Plant Soil 44:525–542

Zimmerman T, Ibrahim SA (2018) Parallel colorimetric quantification of choline and phosphocholine as a method for studying choline kinase activity in complex mixtures. Antibiotics 7:24

Zulfiqar F, Ashraf M (2023) Proline alleviates abiotic stress induced oxidative stress in plants. J Plant Growth Regul 42:4629–4651