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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*

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• *Background and Aims* Different populations of the Mediterranean xerohalophyte species *Atriplex halimus* exhibit different levels of resistance to salt and osmotic stress depending on the nature of the osmocompatible solute they accumulate. There is, however, no conclusive description of the involvement of abscisic acid (ABA) in the plant response to NaCl or osmotic stress in this species.
• *Methods* Seedlings issued from an inland water-stress-resistant population (Sbikha) and from a coastal salt-resistant one (Monastir) were exposed in nutrient solutions to NaCl (40 or 160 mM) or to 15 % PEG for 1 d and 10 d in the presence or absence of 50 μM ABA.
• *Key Results* Plants from Sbikha accumulated higher amounts of ABA in response to osmotic stress than those of Monastir, while an opposite trend was recorded for NaCl exposure. Exogenous ABA improved osmotic stress resistance in Monastir through an improvement in the efficiency of stomatal conductance regulation. It also improved NaCl resistance in Sbikha through an increase in sodium excretion through the external bladders. It is suggested that polyamines (spermidine and spermine) are involved in the salt excretion process and that ABA contributes to polyamine synthesis as well as to the conversion from the bound and conjugated to the free soluble forms of polyamine. Proline accumulated in response to osmotic stress and slightly increased in response to ABA treatment while glycinebetaine accumulated in response to salinity and was not influenced by ABA.
• *Conclusions* It is concluded that ABA is involved in both salt and osmotic stress resistance in the xerohalophyte species *Atriplex halimus* but that it acts on different physiological cues in response to those distinct environmental constraints.

Key words: Abscisic acid, *Atriplex halimus*, bladders, glycinebetaine, halophyte, osmotic stress, polyamines, proline, salinity, saltbush, sodium.

INTRODUCTION

Increased salt and drought tolerance of crops are needed to sustain food production in the future since most of suitable land is already used for agriculture production. There is also an urgent need to reduce the amounts of water used for irrigation not only because fresh water will become less available in the future but also because the use of poor quality water is responsible for salinization of irrigated lands in numerous areas of the world (Gerhart *et al.*, 2006). Tolerance of those abiotic stresses is a complex trait from a genetic and a physiological point of view. Most of the cultivated plant species are rather sensitive to both salt and water stress. Hence, there is an obvious interest in studying the physiological basis of stress resistance in xero-halophyte plant species which are well adapted to salinity and drought. Such an approach may help us to identify the key components of stress resistance and, in a second step, to define precisely their genetic basis with the final aim of transferring such properties to cultivated plant species (Colmer *et al.*, 2006). Beside their putative interest for plant breeding, xero-halophyte plants are also important from an ecological point of view: global change will undoubtedly contribute to desertification processes in the next decades and the optimal use of this plant material may be considered as a convenient way to reduce the deleterious impact of climate change in marginal ecosystems. The efficient use of xero-halophyte plant species could also help in the reduction of global warming through CO_2 sequestration (Glenn *et al.*, 1992). Because some of these species are also able to tolerate high levels of inorganic pollutants in the soil, they may constitute a valuable material for land rehabilitation in former mining areas, especially under arid environments (Lutts *et al.*, 2004). A better knowledge of the physiological behaviour of those species exposed to water stress and/or salinity is therefore of considerable interest.

Plants exposed to salt stress have to face a double constraint: the first one is directly linked to the accumulation of toxic ions (mainly Na^+ and Cl^-), while the other one is related to the decrease in the external soil water potential which compromises water absorption, thus inducing a physiological drought within the stressed tissues (Munns, 2002). Abscisic acid (ABA) is frequently overproduced in response to a wide

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range of environmental constraints. This plant hormone is synthesized in leaves and roots and may move freely through the plant in both the xylem and the phloem. The impact of ABA on stomatal regulation is well established and it may thus contribute to reduce water losses. This hormone has also been reported to be directly responsible for the activation of numerous genes involved in plant response to water stress (Bray, 2002).

Among the known mechanisms associated with stress tolerance, accumulation of osmoprotectants such as glycinebetaine and proline is of particular interest. Those compounds may be involved in both osmotic adjustment and protection of cellular structures. It has been demonstrated that proline and glycinebetaine could reduce the deleterious impact of stress on the generation and/or impact of reactive oxygen species (Hoque *et al.*, 2007), protect enzymes assuming crucial functions in plant metabolism (Rontein *et al.*, 2002) and stabilize the photosystems (Ohnisi and Murata, 2006). Other authors assume that those compounds could also be involved in stress signalling (Ma *et al.*, 2006). Conflicting data are, however, available in the literature concerning the putative influence of ABA on proline and glycinebetaine synthesis (Colmer *et al.*, 2005).

The diamine putrescine (Put) and the polyamines spermidine (Spd) and spermine (Spm) are low molecular weight organic cations that are implicated in various physiological and developmental processes in all living organisms. In plants, these processes include regulation of cell division, rhizogenesis, embryogenesis, senescence, floral development and fruit ripening (Galston *et al.*, 1997; Arena *et al.*, 2005). In addition, polyamines have been shown to afford protection against a large number of environmental biotic and abiotic stresses (Bouchereau *et al.*, 1999; Balestrasse *et al.*, 2005). Under physiological conditions, polyamines may be present in a free soluble form but could also be conjugated to phenolic and hydroxycinnamic acids or bound to macromolecules such as proteins and nucleic acids (Sfichi *et al.*, 2004, Bakhanashvili *et al.*, 2005). ABA has been shown to increase polyamine synthesis in wheat (Aurisano *et al.*, 1993) and rice (Lee *et al.*, 1997). It has also been demonstrated in rice that the ionic component of salt stress may have specific impact on polyamine metabolism and that iso-osmotic concentrations of NaCl on the one hand and polyethylene glycol (PEG) on the other hand do not necessarily have similar effects on polyamine concentrations (Lefèvre *et al.*, 2001). Data concerning polyamine metabolism in xero-halophyte species exposed to drought or to salinity are, however, crucially lacking.

Beside osmotic adjustment and protection of cellular structures, exclusion of the absorbed ions at the leaf surface often appears as an efficient strategy contributing to salinity resistance in some xero-halophte plant species (Batanouny *et al.*, 1992; Ramadan, 1998). This appears to be valid for *Atriplex halimus* since both abaxial and adaxial leaf surfaces are covered by high density of salt-accumulating bladders, also defined in the literature as vesiculated hairs or trichomes (Mozafar and Goodin, 1970; Breckle *et al.*, 1990). According to Freitas and Breckle (1993), >50 % of the absorbed sodium and chloride may be removed from the mesophyll through this mechanism. As far as is known, however, no exhaustive data are available on the influence of ABA on these salt excretion processes.

It has been demonstrated recently that an inland (Sbikha) and a coastal (Monastir) population of *Atriplex halimus* widely differ in terms of physiological strategy allowing the plant to cope with NaCl and osmotic stress (Ben Hassine *et al.*, 2008). The coastal population is more tolerant of salinity than the inland population and displays a higher ability to accumulate glycinebetaine in response to this constraint. In contrast, the inland population, exposed in its natural habitat to transient periods of drought, is more resistant to osmotic stress induced by 15 % PEG, and mainly accumulates proline in response to this treatment (Ben Hassine *et al.*, 2008). The coastal population also exhibits a prodigal use of water in respect of a low instantaneous water-use efficiency (WUE), while the inland population is adopting a water-saving strategy and thus exhibits a high WUE. Finally, the higher salt-resistance of the coastal population is not associated with a lower sodium accumulation when plants are maintained in the presence of 160 mM under controlled conditions (Ben Hassine *et al.*, 2008). The endogenous concentration of ABA, the efficiency of the salt excretion process and the putative influence of ABA on salt excretion were never considered until now.

The aims of the present study were (*a*) to determine the impact of salt and osmotic stress on the endogenous concentration of ABA in plants of *A. halimus* belonging to these two distinct populations and (*b*) to analyse the putative physiological influence of this plant growth regulator on seedling response to stress. It is shown that ABA improved the behaviour of coastal population exposed to osmotic stress by improving its stomatal regulation while it helps the inland population to cope with salinity by stimulating Na^+ and Cl^- exclusion at the bladder level.

MATERIALS AND METHODS

Plant material and growth conditions

Fruits of *Atriplex halimus* L. were collected from wild plants growing at two different sites in Tunisia. Monastir is a coastal site from East-Tunisia (36°13′N; 10°23°W) while Sbikha is an arid inland site (36°27′N; 9°49′W). The mean annual rainfall (average estimated over the previous 3 years) for Monastir and Sbikha are 346 mm and 253 mm, respectively. The mean relative humidity and the mean annual temperature are 72·9 % and 20·0 °C for Monastir and 60·3 % and 23·2 °C for Sbikha. For both sites, an experimental field of approx. 50 ares spontaneously colonized by shrubs of *Atriplex halimus* was considered. The soil salinity level was estimated on ten independent soil samples per site. Soil samples were collected in June (which corresponds to a mean precipitation of 27 mm and 35 mm for Sbikha and Monastir, respectively). Electrical conductivity was measured on saturate paste extracts collected at a 20-cm depth using a WTW LF92 conductimeter. The mean electrical conductivity was $2{\cdot}23 \pm 0{\cdot}43$ dS m^{-1} for Sbikha and $7{\cdot}18 \pm 1{\cdot}03$ dS m^{-1} for Monastir, thus confirming the saline properties of the latter and the non-saline character of the former site. Seeds were collected from at least ten plants at each site and pooled in order to constitute a mean sample for each population.

After removal of the bracts, seeds were sown in plastic jars containing a sandy textured non-saline soil (50 % sand, 25 %

silt, 25 % clay; EC 1·13 dS m^{-1}) and maintained in a growth chamber at 28 °C during the day, 20 °C during the night, under a PAR of 150 μmol m^{-2} s^{-1} and a photoperiod of 16 h. After 5 weeks, 320 seedlings per population were distributed among 40 tanks, each one containing 2 L of 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. Solutions were renewed each week. Plants (eight per tank) were fixed on polystyrene plates at a mean distance of 6 cm. Daytime humidity was maintained at 57 ± 2 % and temperature at 25 °C during the day and 23 °C during the night. The mean PAR was 250 μmol m^{-2} s^{-1} provided by Philips lamps (Philips Lighting S.A., Brussels, Belgium).

Treatments were applied 10 d after transfer to the nutrient solution. For salt treatment, NaCl was added to the nutrient solution in order to obtain a final concentration of 40 or 160 mM as previously recommended (Ben Hassine *et al.*, 2008). For osmotic stress, PEG (polyethylene glycol 10000; Sigma Aldrich, Belgium) was added in nutrient solution to reach a final dose of 15 %. Plants maintained in nutrient solution in the absence of NaCl and PEG were used as a control. Osmotic potentials of nutrient solutions were assessed with a vapour pressure osmometer (Wescor 5500): Ψ_s = −0·12 (control), −0·37 (40 mM NaCl), −1·04 (160 mM NaCl) and −0·97 (15 % PEG) MPa. Solutions were continuously aerated with a stream of air and kept at a constant O_2 concentration of 7·5 mg L^{-1} (87 %) measured with a dissolved oxygen probe (CellOx 325; WTW) and a precision instrument (ProfiLine Dissolved Oxygen Meter Oxi 197-S; WTW). Each of the four treatments was applied on 60 plants per population: 12 of them were harvested after 24 h of exposure and another set of 12 plants after 10 d of treatment. Leaf stomatal conductance (g_{leaf}) was measured daily at 1100 h on the abaxial surface of the leaf located at the middle part of the main stem with an automatic porometer (MK III; Delta-T Devices, UK). For further analysis (ion, ABA, polyamine, proline and glycinebetaine concentration), measurements were performed on a pooled sample of fully expanded leaves collected on a plant, the four basal leaves being discarded to avoid interference with senescing processes. For each treatment, six separated plants were considered.

In another set of experiments, plants were exposed to the same stressing agents in the presence or in the absence of 50 μM ABA (Sigma-Aldrich, Germany). Additions of inhibitors such as 50 μM fluridone (FLU; Olchemlm Ltd, Czech Republic), a known inhibitor of ABA biosynthesis (Kowalczyk-Schröder and Sandmann, 1992) or 0·5 mM methylglyoxal-*bis*-guanyl hydrazone (MGBG; Sigma Aldrich Belgium), an inhibitor of polyamine biosynthesis, were made when required as stated below.

Ion quantification and bladders analysis

For major cations (K^+, Na^+, Mg^{2+} and Ca^{2+}), Pi and microelement (Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{3+}) quantification, tissues harvested on five plants per treatment were oven-dried at 80 °C for 48 h and 50 mg dry weight were digested in 35 % (v/v) HNO_3. Analyses were conducted by flame atomic absorption spectrophotometry (VARIAN spectra-300). Chloride was determined colorimetrically with feric ammonium sulfate and mercuric thiocyanate according to Guerrier and Patolia (1989).

For the scanning electron microscopy (SEM Phillips XL20), specimens were flash frozen (−212 °C) in liquid nitrogen under vacuum for cryo-SEM (Oxford CT1500 cryo-system), transferred to the preparation chamber, and then to the SEM chamber where the frozen samples were sublimated (−80 °C) to remove ice particles. Samples were sputter coated with gold in the preparation chamber for 75 s under 1·2 kV at −150 to −170 °C. Specimens were viewed under − 5 kV at −170 to −190 °C.

Bladders (also termed 'trichomes' or 'vesiculated hairs' in the genus *Atriplex*; Mozafar and Goodin, 1970; Breckle *et al.*, 1990; Freitas and Breckle, 1993) were removed from the leaf surface on samples collected at the mid-photoperiod on eight separated plants per treatment according to Zhang and Oppenheimer (2004) by carefully rubbing the surface of the weighed leaves using a small paintbrush in the presence of 0·05 % Triton X-100 (Sigma Inc; high grade chemical reagent) (the absence of ion contamination in the washing buffer was checked before leaf rinsing by atomic absorption spectrophotometry; Varian SpectrAA-300). The washing solution was retained and stored at −20 °C until ion analysis while the washed leaves were dried in an oven. As far as leaves were concerned, ion quantifications were performed according to Ramadan (1998) on intact leaves, washed leaves and washing solution in order to determine the proportions of ions which were outside (at the leaf surface) and inside (remaining within the leaf tissues).

ABA determination

For ABA quantification, plant tissues were powdered in liquid nitrogen and extracted with 100 % methanol for 2 h at −20 °C in darkness. [^{3}H]ABA (777 TBq mol^{-1}) was added to each sample as a recovery marker at 250 Bq. After centrifugation (24 000 *g* at 4 °C for 20 min), the supernatant was diluted with water to a final concentration of 80 % methanol and purified on a C_{18}SepPack Cartridge (Waters, Ireland). ABA concentration was determined according to Djilianov *et al.* (1994). ABA extracts were evaporated to dryness *in vacuo* and redissolved in phosphate-buffered saline. ABA content was quantified by using ELISA kits (Phytoscience, France) with a monoclonal antibody (Olchemlm Ltd, Czech Republic) specific to free *cis*-(+)-ABA. The accuracy of the immunoassay was checked by LC-MS. The HPLC was coupled to a VG TRIO 2000 quadrupole mass spectrometer with a VG thermospray probe (Fisons, Manchester, UK) under optimized thermospray conditions (repeller 200 V, capillary temperature 205 °C). Quantitative analyses were performed in Selected Ion Monitoring mode using 265 $(MH)^+$ and 247 $(M\text{-}H_2O)H^+$ on specific diagnostic ions.

Polyamines, glycinebetaine and proline extraction and quantification

For polyamine extraction, tissues were frozen in liquid nitrogen and ground in a pre-chilled mortar: samples [approx. 500 mg fresh weight (f. wt) for shoots and 250 mg for roots] were then homogenized with 500 μL of cold HCl (1 M), kept on ice for

1 h and then centrifuged at 23100 *g* at 4 °C for 20 min. Pellets were re-extracted with 500 μL HCl (1 M) and re-centrifuged. The two supernatants were used to determine free polyamines. Dansylation was performed according to Smith and Davies (1985). Samples were re-suspended in 1 mL methanol, centrifuged at 13 000 *g* for 15 min and filtered through microfilters (Chromafil PES-45/15, 0·45 μm; Macherey-Nagel). Aliquots (20 μL) were injected into a Bio-Rad HPLC system equipped with a Nucleosil 100-5 C18 MN 250/04 column (particle size: 5 μm, 4·6 × 250 mm^2). Elution was performed at 35 °C at a flow rate of 1 mL min^{-1} using a methanol/water stepped gradient programme changing from 60 % to 100 % methanol over 25 min. The column was washed with 100 % methanol for 15 min. Detection of dansylated polyamines was performed with a Shimadzu RF-10Axl fluorimeter, with an excitation wavelength of 320 nm and an emission wavelength of 510 nm. The rate of free polyamine recovery at the end of the procedure was higher than 95 % (Ndayirajige, 2006).

For free and conjugated polyamine analysis, 200 μL of the supernatant was mixed with 200 μL of 12 N HCl and heated at 110 °C for 16 h in tightly capped glass tubes. After acid hydrolysis, HCl was evaporated from the tubes by further heating at 80 °C and the residue was resuspended in 200 μL of 10 % PCA and used for dansylation. To extract PCA, insoluble bound PAs, the pellet was dissolved by vigorous vortexing in 5 mL of 1 N NaOH. The mixture was centrifuged at 23 100 *g* at 4 °C for 20 min, and the supernatant, including the solubilized bound PAs, was hydrolysed under the same conditions as above. Aliquots of 200 μL of supernatant (free PAs), supernatant hydrolysed (conjugated PAs) and pellet hydrolysed (bound PAs) were dansylated, along with PAs standards, as previously described by Lefèvre *et al.* (2001). Polyamines were then quantified as described above for free polyamines. For a given treatment, each quantification was performed on three independent samples.

For glycinebetaine determination, collected leaves and roots (approx 200 mg) were mixed with 5 mL distilled water and the crude extracts were applied to a small column (1·6 mL) containing an AG1 X8 resin (200–400 mesh, OH- form Bio-Rad). The column was dried down by centrifugation (3 min, 4 °C, 300 *g*) and then washed with 875 μL of distilled water. Extracted glycinebetaine was quantified according to Bessieres *et al.* (1999) after HPLC separation on a Spherisorb 5 ODS2 column (250 × 4·6 mm) preceded by a precolumn (10 × 1 mm) packed with the same phase. The mobile phase contained 13 mM sodium heptane sulfonic acid and 5 mM Na_2SO_4 in deionized water (pH adjusted to 3·7 with 1 N H_2SO_4) at a flow rate of 0·8 mL min^{-1}. Quantification was performed by a UV detector (Bio-Rad 1801 UV Monitor). The rate of glycinebetaine recovery at the end of the procedure was at least 94 % (Ndayirajige, 2006).

To quantify free proline, 1 g of tissue was extracted with 5 mL of 5 % salycilic acid; after centrifugation at 5000 *g*, free proline was specifically quantified according to Bates *et al.* (1973).

Statistical treatment of the data

The experiment was repeated twice (March and April 2004; April and May 2005) and the results exhibited similar trends. Data presented hereafter are from one single experiment. Percentage data were transformed to arcsine values before statistical analysis. For each duration of stress, data were analysed separately for roots and shoots by one-way analysis of variance (ANOVA, treatment as level of classification) performed with the model procedure of SAS version 9·1 (SAS Institute, 2002). Mean differences were compared according to the Scheffé *F* test.

RESULTS

Moderate doses of salt induced a slight increase in shoot and root dry weights (Fig. 1) in seedlings issued from both populations after 10 d of treatment. Shoot and root dry weight decreased in response to 160 mM NaCl in Sbikha compared with controls but not in Monastir, thus confirming the higher salt resistance of the latter compared with the former. Root and shoot dry weight in Sbikha exposed to 15 % PEG remained unaffected compared with controls. In contrast, dry weight of both organs was strongly affected in plants of Monastir maintained for 10 d in the presence of 15 % PEG.

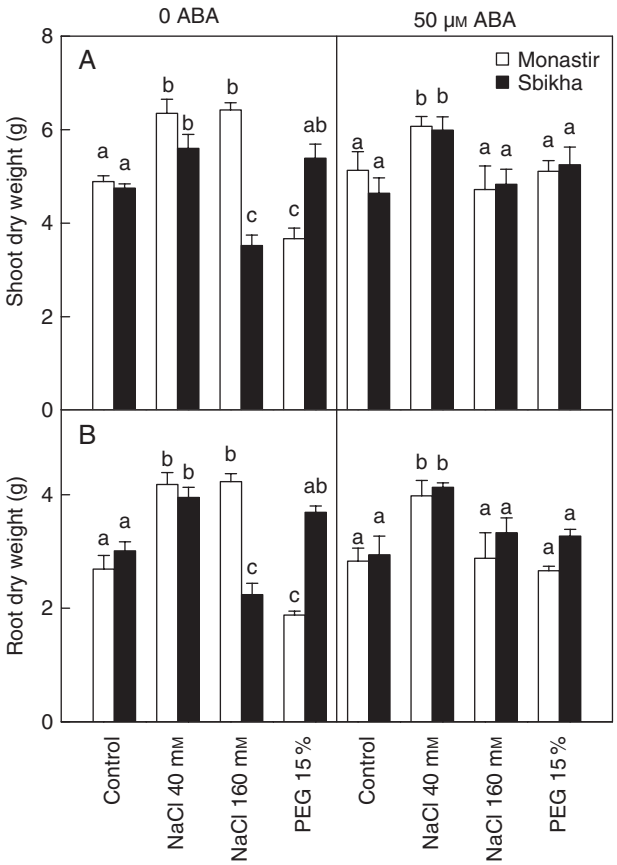

FIG. 1. Shoot and root dry weights in seedlings of *Atriplex halimus* issued from a coastal saline site (Monastir) or an inland semi-arid area (Sbikha) and exposed for 10 d to control nutrient solution or to nutrient solution containing 40 or 160 mM NaCl or 15 % PEG in the absence or presence of 50 μM ABA in the nutrient solution. Initial dry weights were 2·53 ± 0·13 g and 1·97 ± 0·11 g for shoots and roots, respectively. Each value is the mean of 12 replicates and vertical bars are s.e. of the means. Values sharing a common letter are not significantly different at $P < 0·05$.

Addition of ABA 50 μM improved plant growth in Sbikha exposed to 160 mM NaCl and in Monastir exposed to PEG, suggesting that this plant growth regulator has a positive impact in plant resistance to both type of environmental constraints.

Root water content was hardly affected by salinity or PEG (detailed data not shown). Leaf water content was already reduced by PEG after 24 h of treatment in Monastir ($P < 0{\cdot}05$) but not in Sbikha (Table 1). After 10 d of treatment, the leaf WC was reduced in both populations in response to the highest dose of NaCl (160 mM) and to 15 % PEG. Nevertheless, the deleterious impact of NaCl was higher in Sbikha than in Monastir while an inverse trend was recorded for PEG. In the absence of exogenous ABA in the nutrient solution, endogenous ABA had already increased in response to salinity in Monastir and in response to osmotic stress in Sbikha after 24 h of treatment (Fig. 2). The endogenous ABA concentration was always higher in Sbikha than in Monastir in response to osmotic stress ($P < 0{\cdot}01$) while an opposite trend was recorded in salt-treated plants (Fig. 2). It might thus be hypothesized that the endogenous concentration of ABA is a limiting factor for acclimation of seedlings from Sbikha exposed to NaCl as well as for those of Monastir exposed to PEG.

Exogenous application of 50 μM ABA strongly increased total ABA concentration in both roots and shoots, thus suggesting that, when added to the nutrient solution, this plant growth regulator was efficiently absorbed by the roots and translocated to the shoots (Table 2). No difference was detected any more between plants of Sbikha and Monastir, whatever the considered treatment. This confirms that the positive impact of exogenous ABA on treated plants was associated with an increase in the root and shoot total ABA concentrations. Conversely, FLU treatment decreased ABA concentrations in all plant organs including unstressed controls after 10 d of treatment. Exogenous application of ABA increased the leaf WC of Monastir exposed to PEG (Table 2) compared with similar seedlings untreated with ABA (Table 1; $P < 0{\cdot}05$) and values of ABA-treated plants were close to those recorded for unstressed controls. In contrast, exogenous ABA had no impact on the leaf WC of salt-treated Sbikha which remained lower for plants exposed to 160 mM NaCl than for controls.

TABLE 1. *Leaf water content (in %; measured on fully expanded leaves) in seedlings of* Atriplex halimus *issued from a coastal site (Monastir) or an inland area (Sbikha) and exposed for 1 or 10 d to control nutrient solution or to a nutrient solution containing 40 or 160 mM NaCl or 15 % PEG*

Treatment	Population	1 d	10 d
Control	Monastir	$84{\cdot}2 \pm 0{\cdot}7^{a}$	$83{\cdot}9 \pm 1{\cdot}0^{a}$
	Sbikha	$85{\cdot}3 \pm 1{\cdot}3^{a}$	$82{\cdot}1 \pm 0{\cdot}8^{a}$
40 mM NaCl	Monastir	$83{\cdot}9 \pm 0{\cdot}9^{a}$	$82{\cdot}8 \pm 1{\cdot}1^{a}$
	Sbikha	$83{\cdot}4 \pm 1{\cdot}2^{a}$	$81{\cdot}7 \pm 0{\cdot}8^{b}$
160 mM NaCl	Monastir	$81{\cdot}5 \pm 0{\cdot}2^{b}$	$77{\cdot}9 \pm 0{\cdot}4^{bc}$
	Sbikha	$76{\cdot}8 \pm 0{\cdot}4^{c}$	$72{\cdot}5 \pm 1{\cdot}2^{d}$
15 % PEG	Monastir	$77{\cdot}3 \pm 0{\cdot}8^{c}$	$70{\cdot}1 \pm 0{\cdot}4^{e}$
	Sbikha	$84{\cdot}1 \pm 0{\cdot}4^{a}$	$78{\cdot}2 \pm 1{\cdot}1^{c}$

Each value is the mean of six replicates ± s.e. of the means. For a given time, values sharing a common superscript letter are not significantly different at $P < 0{\cdot}05$.

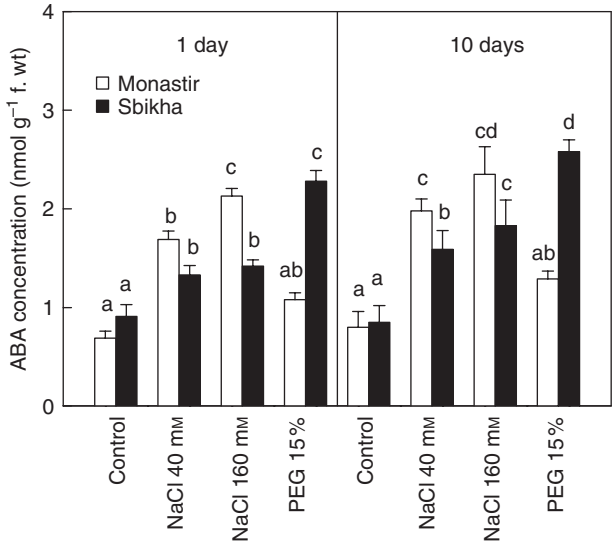

FIG. 2. Leaf abscisic acid concentration (ABA, in nmol g^{-1} f. wt) in seedlings of *Atriplex halimus* issued from a coastal saline site (Monastir) or an inland semi-arid area (Sbikha) and exposed for 1 d and 10 d to control nutrient solution or to nutrient solution containing 40 or 160 mM NaCl or 15 % PEG. Measurements were performed on fully expanded leaves. Each value is the mean of 12 replicates and vertical bars are s.e. of the means. Values sharing a common letter are not significantly different at $P < 0{\cdot}05$.

The g_{leaf} was similar in Sbikha and Monastir under control conditions: after 24 h of exposure to all treatments in Sbikha it had already clearly decreased. After 10 d of stress exposure, g_{leaf} was lower in Sbikha than in Monastir, whatever the considered treatment (Fig. 3). Exogenous ABA decreased g_{leaf} in PEG-treated plants issued from Monastir but had no impact on salt-treated plants from both populations (Table 2). Conversely, FLU treatment increased g_{leaf} in water-stressed plants ($P < 0{\cdot}01$) but had only a limited impact on g_{leaf} from salt-treated individuals.

Sodium and chloride concentrations in the shoots of control plants were similar in Sbikha and in Monastir (Fig. 4). Low (40 mM) and high (160 mM) doses of NaCl induced an obvious increase in those elements which accumulated to higher extent in Monastir than in Sbikha ($P < 0{\cdot}05$). Sodium concentration also slightly (although significantly) increased in response to PEG in both populations while K^+ concentration decreased in salt-treated seedlings from Sbikha only. Other ion concentrations were not modified, whatever the considered treatment or population (data not shown). Exogenous application of ABA had only a marginal impact on total Na^+ and Cl^- concentrations of stressed seedlings (data not shown) but it drastically affected ions distribution among bladders and lamina. Indeed, a high density of bladders is visible at the leaf surface (Fig. 5). Those vesiculated hairs consist of a stalk cell and a balloon-like tip cell which sequester excess electrolyte and release the salt back in the environment when they are ruptured. Because of the presence of salt crystals at their surface, leaves of *Atriplex halimus* have a silver reflectance and this layer of bladders is thus an efficient system to

TABLE 2. *Impact of exogenous ABA (50 μM) and fluridone (FLU) application on total leaf ABA concentration, water content (WC) and leaf stomatal conductance (g_{leaf}) in seedlings of* Atriplex halimus *issued from a coastal site (Monastir) or an inland area (Sbikha) and exposed for 10 d to control nutrient solution or to a nutrient solution containing 40 or 160 mM NaCl or 15 % PEG*

		Total ABA (nmol g^{-1} f. wt)		WC (in %)		g_{leaf} (cm s^{-1})	
	Population	50 μM ABA	FLU	50 μM ABA	FLU	50 μM ABA	FLU
Control	Monastir	$5{\cdot}13 \pm 0{\cdot}12^{a}$	$0{\cdot}21 \pm 0{\cdot}05^{a}$	$85{\cdot}6 \pm 1{\cdot}1^{a}$	$77{\cdot}3 \pm 0{\cdot}8^{a}$	$0{\cdot}27 \pm 0{\cdot}01^{a}$	$0{\cdot}39 \pm 0{\cdot}04^{a}$
	Sbikha	$5{\cdot}02 \pm 0{\cdot}22^{a}$	$0{\cdot}35 \pm 0{\cdot}02^{ab}$	$84{\cdot}5 \pm 0{\cdot}9^{a}$	$78{\cdot}4 \pm 1{\cdot}1^{a}$	$0{\cdot}24 \pm 0{\cdot}03^{a}$	$0{\cdot}36 \pm 0{\cdot}03^{a}$
40 mM NaCl	Monastir	$5{\cdot}27 \pm 0{\cdot}14^{a}$	$0{\cdot}29 \pm 0{\cdot}02^{a}$	$84{\cdot}9 \pm 1{\cdot}3^{a}$	$73{\cdot}2 \pm 1{\cdot}0^{b}$	$0{\cdot}25 \pm 0{\cdot}02^{a}$	$0{\cdot}27 \pm 0{\cdot}03^{b}$
	Sbikha	$4{\cdot}89 \pm 0{\cdot}18^{a}$	$0{\cdot}25 \pm 0{\cdot}08^{a}$	$80{\cdot}5 \pm 0{\cdot}8^{a}$	$69{\cdot}5 \pm 0{\cdot}4^{c}$	$0{\cdot}19 \pm 0{\cdot}01^{b}$	$0{\cdot}23 \pm 0{\cdot}01^{b}$
160 mM NaCl	Monastir	$5{\cdot}12 \pm 0{\cdot}23^{a}$	$0{\cdot}42 \pm 0{\cdot}04^{b}$	$82{\cdot}3 \pm 1{\cdot}7^{a}$	$75{\cdot}4 \pm 1{\cdot}2^{ab}$	$0{\cdot}30 \pm 0{\cdot}02^{a}$	$0{\cdot}29 \pm 0{\cdot}02^{b}$
	Sbikha	$4{\cdot}94 \pm 0{\cdot}38^{a}$	$0{\cdot}25 \pm 0{\cdot}03^{a}$	$75{\cdot}3 \pm 2{\cdot}4^{b}$	$67{\cdot}4 \pm 1{\cdot}3^{c}$	$0{\cdot}20 \pm 0{\cdot}03^{ab}$	$0{\cdot}27 \pm 0{\cdot}03^{b}$
15 % PEG	Monastir	$4{\cdot}98 \pm 0{\cdot}27^{a}$	$0{\cdot}29 \pm 0{\cdot}07^{a}$	$84{\cdot}9 \pm 0{\cdot}7^{a}$	$70{\cdot}2 \pm 0{\cdot}5^{bc}$	$0{\cdot}11 \pm 0{\cdot}02^{c}$	$0{\cdot}49 \pm 0{\cdot}03^{c}$
	Sbikha	$5{\cdot}05 \pm 0{\cdot}15^{a}$	$0{\cdot}45 \pm 0{\cdot}02^{b}$	$83{\cdot}1 \pm 1{\cdot}1^{a}$	$74{\cdot}5 \pm 1{\cdot}7^{b}$	$0{\cdot}12 \pm 0{\cdot}02^{c}$	$0{\cdot}52 \pm 0{\cdot}05^{c}$

Measurements were performed on fully expanded leaves. Each value is the mean of six replicates ± s.e. of the means. For a given parameter and a given time, values sharing a common superscript letter are not significantly different at $P < 0{\cdot}05$.

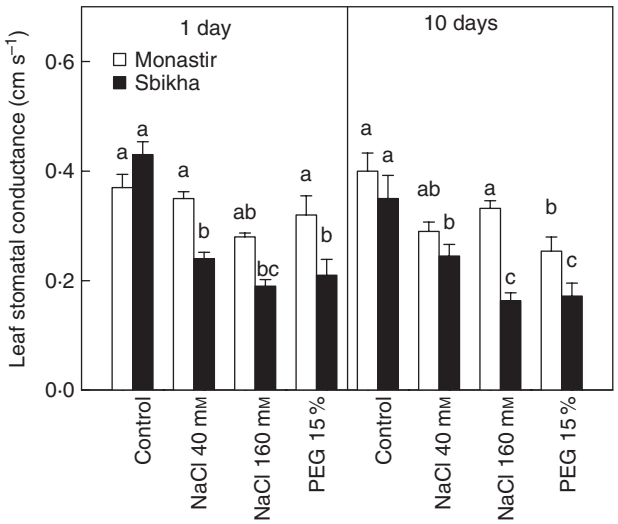

FIG. 3. Leaf stomatal conductance (g_{leaf}, in cm s^{-1}) in seedlings of *Atriplex halimus* issued from a coastal saline site (Monastir) or an inland semi-arid area (Sbikha) and exposed for 10 d to control nutrient solution or to nutrient solution containing 40 or 160 mM NaCl or 15 % PEG. Measurements were performed at 1100 h on a fully expanded leaf located at the middle part of the stem. Each value is the mean of 12 replicates and vertical bars are s.e. of the means. Values sharing a common letter are not significantly different at $P < 0{\cdot}05$.

remove salt from photostynthetically active tissues and probably to prevent some ultraviolet light reaching leaf tissues. As shown in Table 3, it is noteworthy that salt-treated seedlings of Monastir excreted a higher proportion of Na^+ and Cl^- at the leaf surface than seedlings issued from Sbikha and that the difference between populations increased with the NaCl dose. As far as K^+ is concerned, <15 % was found in the bladders and K^+ excretion was not influenced by the treatment and did not differ between populations. It has also to be noticed that exogenous ABA which improved salinity tolerance in Sbikha also clearly improved the sodium excretion process in salt-treated seedlings from this population (detailed data not shown). These data thus suggest that the positive impact of ABA in the salt resistance of *Atriplex halimus* could be due to its positive action on toxic ions excretion. This hypothesis was reinforced by the observation that the plants from both populations exposed to FLU were clearly less able to excrete sodium compared with non-treated ones (Table 3). Impact of FLU appeared to be similar, although less marked, on Cl^- distribution.

Salt treatment and osmotic stress induced a significant increase in the shoot polyamine concentration (Fig. 6). As far as salt-treated plants are concerned, concentrations of free Spd and Spm were higher in Monastir than in Sbikha while no difference was recorded for PEG-treated plants. Exogenous treatment with 50 μM ABA induced an increase in Spd and Spm, especially in Sbikha exposed to both NaCl doses, and it also induces a conversion from the conjugated and bound forms of polyamine to the free polyamine form. In order to test the hypothesis that the positive effect of exogenous ABA on salt excretion could be related to polyamine metabolism, plants from a separate set of experiments were treated with 0·5 mM MGBG (inhibitor of *S*-adenosylmethionine decarboxylase; EC 4·1·1·50). As shown in Table 4, MGBG efficiently decreased total Spd and Spm concentration and increased endogenous total Put content. Based on the shoot dry weight, treatment with MGBG decreased the salt resistance of the two considered populations and this effect was directly related to an inhibition of the sodium exclusion process. Treatment with MGBG strongly mitigated the beneficial impact of ABA on the salinity resistance and sodium excretion process in seedlings from the salt-sensitive population Sbikha (Table 4). However, it did not prevent ABA accumulation in the leaf tissue since ABA concentrations in plants simultaneously exposed to ABA and MGBG culminated up to 5·32 and 5·54 nmol g^{-1} f. wt in Monastir and Sbikha, respectively. These data therefore suggest that Spd and Spm could assume key roles in the sodium excretion process triggered by ABA. In contrast, application of the inhibitor of polyamine synthesis MGBG had no impact on the g_{leaf} of PEG-treated plants and did not prevent stomatal closure in ABA-treated plants (Table 4). Similarly, MGBG had no impact on ABA concentration in plant tissues (detailed data not shown).

Both proline and glycinebetaine accumulated in response to salt and osmotic stress (Fig. 7). Proline, however, accumulated to a higher extent in PEG-treated plants than in salt-treated

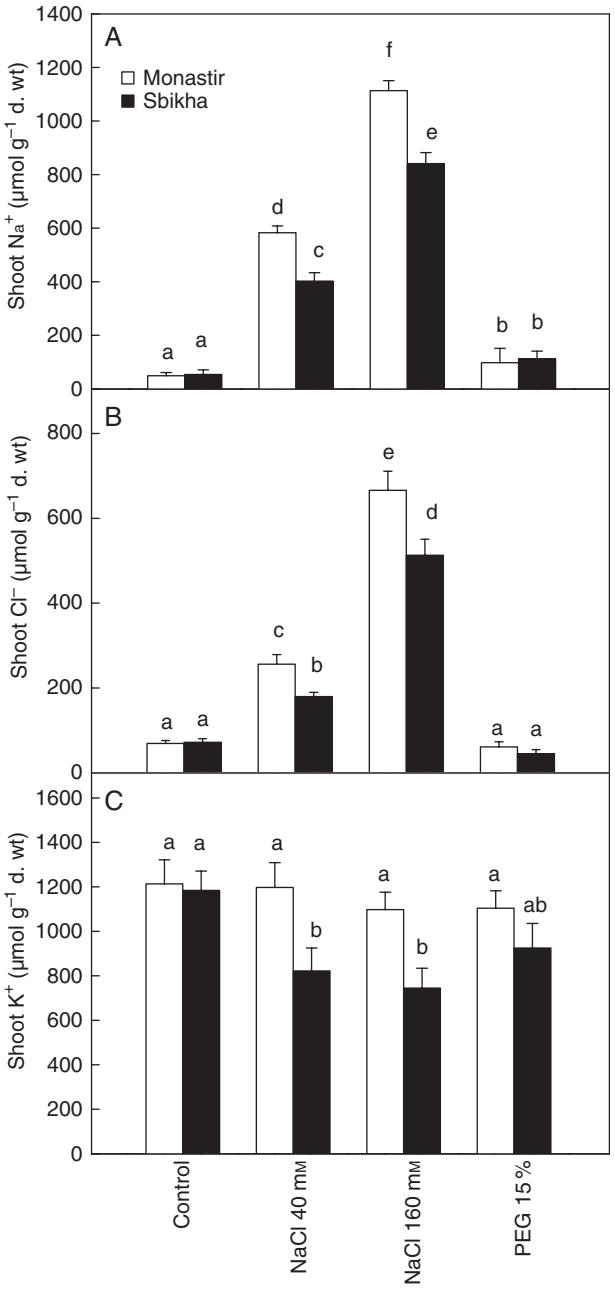

FIG. 4. Sodium (A), chloride (B) and potassium (C) concentrations in the leaves of *Atriplex halimus* issued from a coastal saline site (Monastir) or an inland semi-arid area (Sbikha) and exposed for 10 d to control nutrient solution or to nutrient solution containing 40 or 160 mM NaCl or 15 % PEG in the absence or presence of 50 μM ABA in the nutrient solution. Measurements were performed on fully expanded leaves. Each value is the mean of 12 replicates and vertical bars are s.e. of the means. Values sharing a common letter are not significantly different at $P < 0{\cdot}05$.

ones and reached a higher concentration in Sbikha than in Monastir. Exogenous ABA increased the proline concentration, especially in plants issued from Monastir and exposed to osmotic stress (Fig. 7) while treatment with FLU reduced it in all plants by >40 % (data not shown). In contrast, glycinebetaine reached higher levels in salt-treated plants compared with plants exposed to PEG. At the highest NaCl dose, the leaf glycinebetaine concentration was higher in Monastir than in Sbikha. Exogenous ABA had no impact on glycinebetaine concentration, whatever the treatment or the population considered.

DISCUSSION

Halophye plant species are well adapted to saline habitats and domestication of those species as new crops could in the future help to sustain food production in many regions of the world (Colmer *et al.*, 2005). Salinity affects almost every aspect of plant physiology and biochemistry. Since the presence of salt in the root medium reduces external osmotic potential and thus compromises water absorption, halophyte plant species are exposed in their natural habitats to both ion toxicities and to physiological drought (Flowers and Colmer, 2008). Some xero-halophyte plant species display a fascinating ability to survive in dry and highly saline habitats.

The present data show that ABA is assuming key functions in *Atriplex halimus* in relation to NaCl on the one hand and to osmotic stress on the other. Indeed, the plant performance in the harsh environment appeared to be directly limited by their ability to synthesize this plant growth regulator. The present work suggests that ABA synthesis could be differently affected by environmental parameters depending on the considered population: salt-resistant plants originating from a coastal area accumulated lower amounts of ABA in response to drought than plants issued from an inland area. Environmental and developmental signals are operating in the regulation of ABA synthesis in plant tissues. The direct C15 precursor of ABA xanthoxin is produced as a result of 9-*cis*-violaxanthin cleavage by 9-*cis*-epoxycarotenoid dioxygenase (NCED) which has been established as the key enzyme in the ABA biosynthesis pathway (Xiong and Zhu, 2003). It has been demonstrated that water stress increased expression of the *NCED* gene in glycophyte plant species (Qin and Zeevaart, 2002; Xiong and Zhu, 2003). Some aldehyde oxidases may also oxidize abscisic aldehyde to ABA. Some of them were reported to be activated by drought (Bittner *et al.*, 2001) but not by salinity (Zdunek-Zastoca, 2008). Since both NCED and aldehyde oxidases comprise small multigene families, it is possible that different isoforms are regulated differently by environmental cues in distinct populations. Ren *et al.* (2007) also recently reported that not only ABA synthesis but also catabolism plays a crucial role in the regulation of ABA accumulation by stressed plant tissues. Further studies are therefore required to identify the key limiting factor of ABA synthesis in plants from Monastir exposed to osmotic stress and in those from Sbikha exposed to NaCl. Not only the endogenous concentration of ABA but also the physiological consequences of such an accumulation differed between osmotic stress and salinity. For a given kind of stress, however, the physiological consequences were similar for the two considered populations.

It appears clearly that ABA contributes to osmotic stress resistance through an improvement in stomatal regulation and, hence, to an increased WUE. Plants from Sbikha exposed to water deficit displayed a lower g_{leaf} than those from Monastir and also accumulated higher amounts of ABA in response to PEG. When exogenous ABA was supplied to

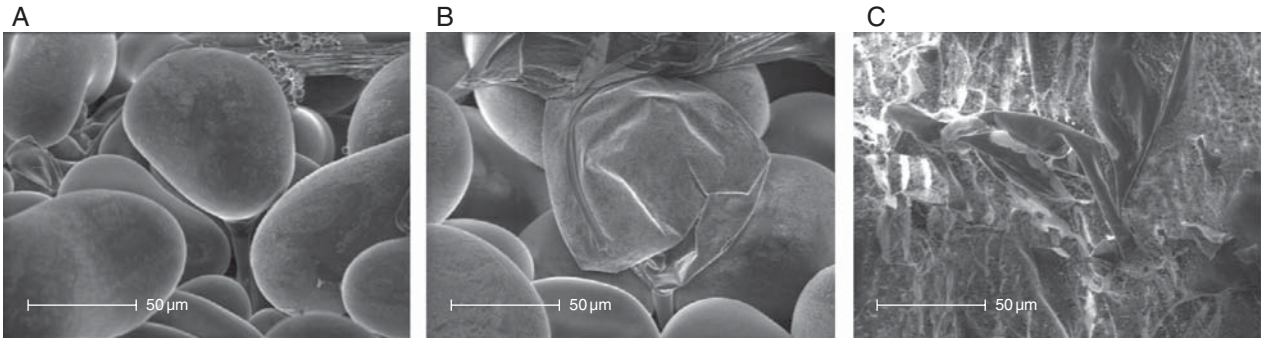

FIG. 5. Scanning electron micrography of the leaf surface of *Atriplex halimus* showing bladders (A) consisting of vesiculated balloon-like hairs attached to a stalk and playing a significant role in removing salt from the reminder of the leaf thus preventing accumulation of toxic salts in the parenchyma and vascular tissues. Accumulation of electrolytes leads to the bladder shrinking (B) and then bursting, depositing crystals at the leaf surface (C).

TABLE 3. *Proportions (in %) of Na^+ and Cl^- excreted at the leaf surface ('Out') or remaining inside the lamina ('In') in the leaves of* Atriplex halimus *issued from a coastal site (Monastir) or an inland area (Sbikha) and exposed for 10 d to control nutrient solution or to a nutrient solution containing 40 or 160 mM NaCl or 15 % PEG*

		Na^+ (distribution in %)						Cl^- (distribution in %)					
		0 ABA		+ ABA		+ FLU		0 ABA		+ ABA		+ FLU	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Control	Monastir	68·8	31·2	67·0	33·0	82·2	17·8	71·5	28·5	64·1	35·9	77·1	22·9
	Sbikha	74·7	25·3	66·2	33·8	78·7	21·3	70·6	29·4	70·8	29·2	84·4	15·6
40 mM NaCl	Monastir	58·6	41·4	55·8	44·2	79·8	20·2	62·8	37·2	59·7	40·3	72·7	27·3
	Sbikha	70·2	29·8	59·6	40·4	84·5	15·5	79·7	20·3	61·6	38·4	82·5	17·5
160 mM NaCl	Monastir	49·7	50·3	48·5	51·5	71·7	28·3	54·2	45·8	50·8	49·2	73·9	26·1
	Sbikha	69·1	30·9	51·7	48·3	76·1	23·9	77·5	22·5	58·7	41·3	79·7	20·3

Plants were maintained in the absence (0 ABA) or in the presence (+ ABA) of 50 µM ABA or 50 µM fluridone (+ FLU) in the nutrient solution. Measurements were performed on fully expanded leaves. Each value is the mean of six replicates.

plants from Monastir, water-stressed plants were able to increase their WUE, thus adopting a water-saving strategy similar to the strategy adopted by the plants from Sbikha and which helps them to cope with water scarcity, even for long periods (Ben Hassine *et al.*, 2008).

Although NaCl implies a lowering of the external water potential and consequently induces a water stress component in the plant tissues, it is noteworthy that WUE did not appear as a key property in salt-treated plants. In contrast, bladders play a significant role. These non-glandular vesiculated hairs present at the leaf surface may accumulate important amounts of Na^+ and Cl^-, thus helping to preserve the metabolic activity of the photosynthethic tissues (Aslam *et al.*, 1986; Karimi and Ungar, 1989). As far as *Atriplex halimus* is concerned, bladders may also accumulate other toxic ions such as Cd^{2+} and Zn^{2+} and the efficiency of the excretion process is partly controlled by environmental factors (Lefèvre *et al.*, 2009).The present data suggest that ABA could be involved in the signalling components controlling excretion. Indeed, exogenous ABA increased Na^+ and Cl^- excretion in salt-treated plants from Sbikha. In contrast, the inhibitor of ABA synthesis, FLU, reduced ABA concentration and the efficiency of the excretion process in both populations.

Osmotic stress was shown to increase Na^+ absorption in *Atriplex halimus*, even in the absence of salt (Martínez *et al.*, 2005) and the PEG-induced increase in shoot Na^+ concentration was therefore not unexpected, although the physiological role of such an increase remains obscure, especially considering that bladders excrete >30 % of the absorbed Na^+. Drought was shown to increase non-glandular bladder density in some species (Gonzáles *et al.*, 2008). Although bladders numbers were not considered in the present study, it has to be mentioned that PEG did not increase the proportion of excreted Na^+, even if ABA obviously increased in the PEG-treated plants. Conversely, exogenous ABA decreased g_{leaf} in PEG-treated plants issued from Monastir but had no impact on the g_{leaf} of salt-treated plants from both populations. These data therefore suggest that ABA may be compartmented to specific cell types depending on the nature of the environmental constraint (stomatal guard cells in water-stressed plants and bladders in salt-treated ones) or that the signal transduction initiated by ABA accumulation is differently involved in the regulation of downstream metabolic processes in those cell types. Another intriguing observation is that not only endogenous ABA but also exogenously supplied ABA appears to be transported to the correct cellular target depending on the nature of environmental constraint.

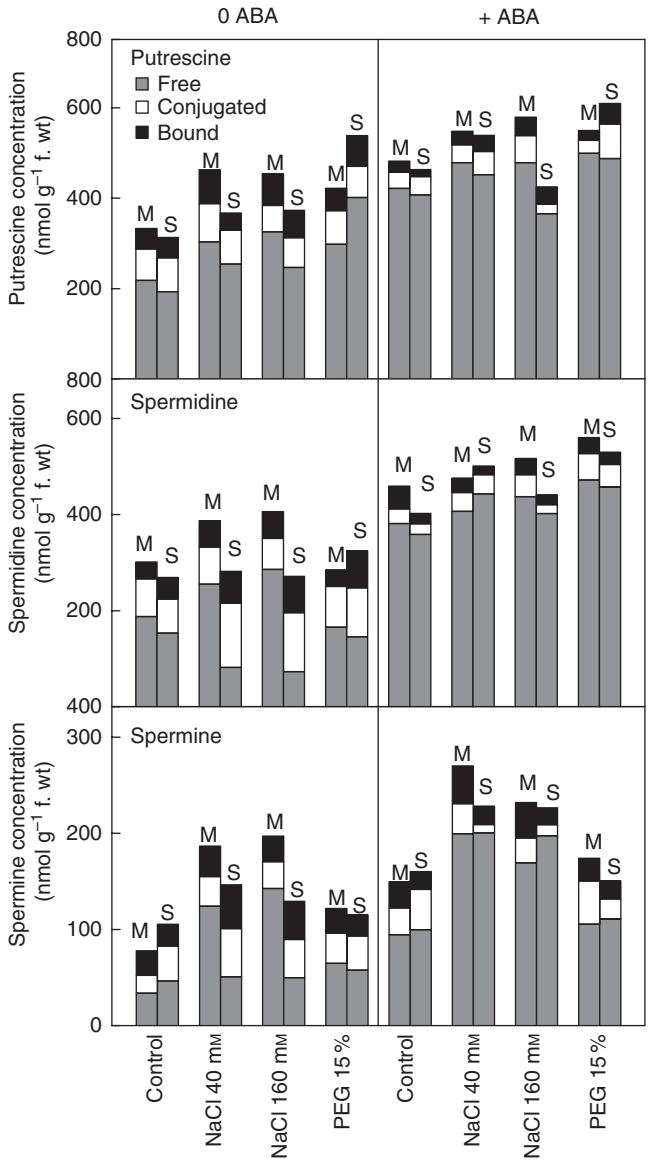

FIG. 6. Leaf polyamine (Putrescine, Spermidine and Spermine) concentration in seedlings of *Atriplex halimus* issued from a coastal saline site (Monastir; M) and an inland semi-arid area (Sbikha; S) and exposed for 10 d to control nutrient solution or to nutrient solution containing 40 or 160 mM NaCl or 15 % PEG in the absence or presence of 50 μM ABA in the nutrient solution. Measurements were performed on fully expanded leaves. Each value is the mean of 12 replicates. In the columns, concentrations are given for the free, conjugated and bound fractions of each polyamine, as indicated.

The data obtained also suggest that free polyamines may be involved in ABA-induced increase of excretion processes through epidermal bladders. Indeed, (*a*) salt-treated seedlings from Monastir contained higher amounts of total polyamines than salt-treated plants from Sbikha, (*b*) exogenous ABA induced an obvious increase in total polyamine content and (*c*) the inhibitor of polyamine synthesis (MGBG) reduced both Spd and Spm concentrations as well as the efficiency of excretion processes. ABA has been reported to trigger polyamine synthesis through a transcriptional activation of genes coding for spermidine synthase (Jiménez-Bremont *et al.*, 2007). Although water stress stimulated both spermidine and spermine synthases in *Arabidopsis thaliana* (Alcázar *et al.*, 2006), no significant PEG-induced increase in Spd and Spm was noticed in *Atriplex halimus* (Fig. 6) despite the fact that Put accumulated in these water-stressed plants. Conversely, Put was shown to control the ABA level in *A. thaliana* (Cuevas *et al.*, 2008) but in *Atriplex halimus*, MGBG-induced increase in Put content did not lead to ABA accumulation, thus suggesting that polyamine metabolism is regulated differently in the Mediterranean xerohalophyte shrub on the one hand and in the model plant species on the other hand.

As far as is known, specific interactions between polyamines and the excretion process at bladder level have never been reported until now. The present data suggest, however, that Spd and Spm rather than Put are involved in this process. Polyamines have been reported to interact with ion fluxes at the tonoplast (Liu *et al.*, 2004; Zhao and Qin, 2004) and plasmamembrane level (Roy *et al.*, 2005). We may thus hypothesize that Spd and Spm could interact with ATPase or ion channels involved in Na^+ and Cl^- fluxes between epidermis and basal cells of bladders. In most cases, however, data available in the literature suggest that regulation of ion fluxes at the membrane level involve the conjugated and bound forms of polyamines in glycophyte species. The present study, however, suggests that free polyamine are more suitable candidates for such regulation in *Atriplex halimus* and that ABA which stimulates salt excretion also increases the free to bound and conjugated ratio of polyamines in this species, as was recently reported in *Vigna vinifera* (Antolín *et al.*, 2008). Shabala *et al.* (2007) recently reported that externally applied polyamines may block a non-selective cation channel from salt-treated pea mesophyll cells, thus preventing Na^+ build-up and K^+ efflux. It is thus also possible that elevated polyamine levels in *Atriplex halimus* leaves reduce Na^+ influx into mesophyll cells thus re-directing Na^+ flux towards salt-bladders.

The present study confirms that glycinebetaine is mainly involved in the response to salinity while proline is involved in the response to water stress, as previously demonstrated in this species (Ben Hassine *et al.*, 2008). ABA has a dual impact on osmocompatible solute accumulation. Indeed, ABA had no significant impact on glycinebetaine concentration while it increased proline synthesis but in water-stressed plants only (Fig. 7). These observations suggest that a specific component of the signal transduction pathway leading to ABA-dependent proline accumulation should be present mainly in response to osmotic stress but not in response to salinity. This unidentified component should be equally present in the two populations considered since PEG-treated seedlings from Sbikha and Monastir accumulated similar amounts of proline in response to exogenous ABA (Fig. 7). Verslues and Bray (2006) using ABA-insensitive and ABA-deficient mutant suggested that sugar sensing may directly influence the efficiency of ABA-induced proline accumulation in water-stressed plants and further studies are therefore required to analyse salt and water stress impacts on sugar metabolism in different populations of *Atriplex halimus*. In some halophytes, leaves have been shown to accumulate high amounts of Na^+ and Cl^-,

TABLE 4. *Impact of methylglyoxal-bis-guanyl hydrazone 0·5 mM (MGBG) on shoot dry weight, total putrescine (Put), total spermidine (Spd) + spermine (Spm) concentration, percentage of sodium excreted at the leaf surface through the bladders [Na (%)] and stomatal conductance (g_{leaf}) in seedlings of* Atriplex halimus *issued from a coastal site (Monastir) and an inland area (Sbikha) and exposed for 10 d to control nutrient solution or to a nutrient solution containing 40 or 160 mM NaCl or 15 % PEG*

		Dry weight (g)			Put (nmol g^{-1} f. wt)			Spd + Spm (nmol g^{-1} f. wt)			Na (%)			g_{leaf} (cm s^{-1})		
	ABA:	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+
	MGBG:	−	+	+	−	+	+	−	+	+	−	+	+	−	+	+
Control	Monastir	4·3	3·9	4·1	351	518	522	418	210	232	30·4	20·6	17·6	0·39	0·43	0·25
	Sbikha	4·1	4·0	3·7	333	507	493	352	173	188	26·5	19·6	15·3	0·42	0·39	0·24
40 mM NaCl	Monastir	5·5	4·0	3·9	434	559	535	607	183	194	39·7	15·4	17·5	0·41	0·44	0·30
	Sbikha	5·2	3·3	3·4	312	498	548	512	179	203	25·8	16·2	20·2	0·25	0·30	0·20
160 mM NaCl	Monastir	6·0	2·8	3·2	429	513	555	594	202	188	49·9	17·3	21·4	0·32	0·35	0·19
	Sbikha	3·1	2·7	3·1	322	497	527	488	161	169	35·6	12·5	18·5	0·21	0·19	0·17
PEG 15 %	Monastir	3·9	4·2	4·0	411	599	603	422	152	225	40·2	20·6	24·6	0·40	0·38	0·14
	Sbikha	5·7	5·2	5·5	421	572	574	434	158	187	34·8	22·9	17·3	0·21	0·24	0·13

Plants were maintained in the presence or absence of 50 μM ABA. Measurements were performed on fully expanded leaves. Each value is the mean of six replicates.

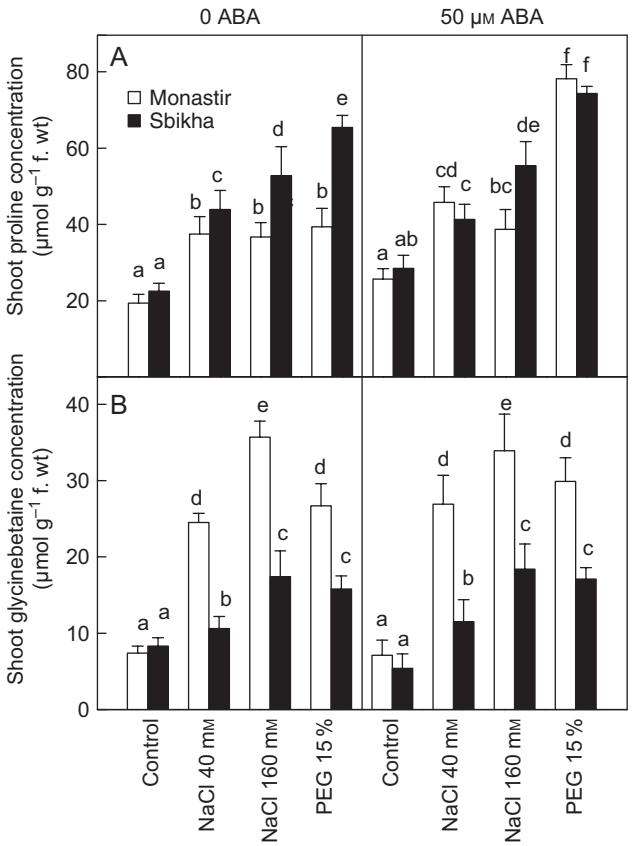

FIG. 7. Leaf proline and glycinebetaine concentrations in seedlings of *Atriplex halimus* issued from a coastal saline site (Monastir) and an inland semi-arid area (Sbikha) and exposed for 10 d to control nutrient solution or to nutrient solution containing 40 or 160 mM NaCl or 15 % PEG in the absence or presence of 50 μM ABA in the nutrient solution. Measurements were performed on fully expanded leaves. Each value is the mean of 12 replicates. Values sharing a common letter are not significantly different at $P < 0{\cdot}05$.

compartmentalizing these ions to vacuoles which then lower the osmotic potential of cells under saline conditions (Song *et al.*, 2009). Plants issued from the population of Monastir had previously been shown to display a higher ability to adjust osmotically in the presence of salt than those from Sbikha (Ben Hassine *et al.*, 2008). Both Na^+ and Cl^- accumulated to higher extent in the former than in the latter (Fig. 4) but a consistent proportion of the accumulated ions were excreted to the external salt bladders (Table 3) where they did not assume crucial osmoprotective functions for stressed tissues. The other quantified ions did not vary in response to salt in Monastir while K^+ even decreased in salt-treated plants from Sbikha. Similarly, PEG had no impact on ion content, beside a slight increase in Na^+ concentrations. It could thus be concluded that organic compounds could assume a key function in the osmotic adjustment of *Atriplex halimus*, even if NO_3^- which could also plays a role in this respect (Song *et al.*, 2009), still has to be quantified.

The present work shows that ABA is involved in both salt and osmotic stress response in the halophyte plant species *Atriplex halimus* but targets different physiological processes in response to the two types of environmental constraints. ABA contributes to osmotic stress resistance through an improvement of stomatal regulation and an increased WUE. In the presence of NaCl, ABA increases excretion of Na^+ and Cl^- in the external salt-bladders and free polyamine appear to be involved in this process.

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