An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress

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Abstract

Soil salinity and drought compromise water uptake and lead to osmotic adjustment in xero-halophyte plant species. These important environmental constraints may also have specific effects on plant physiology. Stress-induced accumulation of osmocompatible solutes was analysed in two Tunisian populations of the Mediterranean shrub *Atriplex halimus* L.—plants originating from a salt-affected coastal site (Monastir) or from a non-saline semi-arid area (Sbikha)—were exposed to nutrient solution containing either low (40 mM) or high (160 mM) doses of NaCl or 15% polyethylene glycol. The low NaCl dose stimulated plant growth in both populations. Plants from Monastir were more resistant to high salinity and exhibited a greater ability to produce glycinebetaine in response to salt stress. Conversely, plants from Sbikha were more resistant to water stress and displayed a higher rate of proline accumulation. Proline accumulated as early as 24 h after stress imposition and such accumulation was reversible. By contrast, glycinebetaine concentration culminated after 10 d of stress and did not decrease after the stress relief. The highest salt resistance of Monastir plants was not due to a lower rate of Na⁺ absorption; plants from this population exhibited a higher stomatal conductance and a prodigal water-use strategy leading to lower water-use efficiency than plants from Sbikha. Exogenous application of proline (1 mM) improved the level of drought resistance in Monastir plants through a decrease in oxidative stress quantified by the malondialdehyde concentration, while the exogenous application of glycinebetaine improved the salinity resistance of Sbikha plants through a positive effect on photosystem II efficiency.

Key words: *Atriplex halimus*, glycinebetaine, halophyte, NaCl, osmotic adjustment, proline, salinity, water stress.

Introduction

Water deficit constitutes a serious threat for agricultural productivity throughout the world. This stress is not only the consequence of a lack of water in the soil but may also be induced by other deleterious environmental conditions such as salinity and other environmental toxicities which compromise the ability of the plant to take up and to translocate water. Some plants, however, have evolved various protective mechanisms allowing them to survive and grow in those harsh environments and respond to water stress through multiple physiological mechanisms. To face the challenge of increasing water demand for agriculture, selection of plants resistant to water deficit is...
of paramount importance and the identification of physiological properties used by xero-halophyte species to cope with salt and/or drought is therefore of great interest (Chaves and Oliveira, 2004; Yamaguchi and Blumwald, 2005; Sambatti and Caylor, 2007).

Osmotic adjustment is defined as the lowering of osmotic potential (\(\Psi_o\)) in plant tissue due to net accumulation of solute. Compatible solutes involved in such processes may act as cytoplasmic osmolytes facilitating water uptake and retention but also as protectors and stabilizers of macromolecules and cellular structures (Bohnert and Jensen, 1996). These beneficial impacts have been reported for proline (Raymond and Smirnoff, 2002), glycinebetaine (Rhodes and Hanson, 1993), soluble sugars, and polyols (Tattini et al., 1996; Sun et al., 1999). It has been considered that there is a relationship between the identity of osmolytes used for osmotic adjustment and the taxonomic position of the considered plant species (Yancey et al., 1982; Hanson et al., 1994). Some plants are unable to synthesize glycinebetaine (Rhodes and Hanson, 1993), while this quaternary ammonium compound is produced in high amounts in plants belonging to the Chenopodiaceae family (McCue and Hanson, 1990). Some recent data, however, suggest that plants able to produce glycinebetaine may also use other compatible solutes for osmoprotection (Di Martino et al., 2003), but the relative contribution of these various compounds to the total osmotic adjustment in relation to the intensity and kinetics of stress exposure is still a matter of debate.

Although both drought and salinity induce water deficit at cellular, tissue, and whole plant levels, they may also have distinct effects on plant metabolism (Yu and Rengel, 1999; Munns, 2002; Hu et al., 2007; Teixeira and Pereira, 2007). This could be especially the case for osmotic adjustment since ions absorbed in excess may themselves contribute to lower the internal \(\Psi_o\) both in halophytes (Zhao and Harris, 1992) and in glycophytes (Cerda et al., 1995). In the absence of salt, K assumes important functions in cellular osmotic regulation, but sodium, being considered as a toxic ion, is thought to accumulate mainly in vacuoles, especially in salt-resistant plants (Zhu, 2000). Modalities of osmotic adjustment, as well as the nature and cellular localization of compounds involved in this process may differ under drought and salt stress, although data concerning the behaviour of distinct populations belonging to the same species but adapted to contrasting stressing environment are scarce.

*Atriplex halimus* L. (Chenopodiaceae) is a monoecious C\(_4\) perennial shrub native to the Mediterranean Basin with an excellent tolerance to drought and salinity. The species is present in semi-arid to subhumid areas of the north Mediterranean and in arid zones from North Africa and the eastern Mediterranean (Le Houérou, 1992). *Atriplex halimus* displays a high level of variability both between and within populations (Ortiz-Dorda et al., 2005; Walker et al., 2005). It may therefore be hypothesized that inland populations exposed mainly to drought could exhibit distinct resistance mechanisms compared with coastal populations which have to face high levels of salts in their substrate. In the specific case of *A. halimus*, however, the relationship between salt and water stress should be considered with attention since NaCl has recently been shown to contribute to water stress resistance (Martínez et al., 2005). Low to moderate levels of salinity have also been shown to stimulate plant growth in this species, and the presence of NaCl should therefore not necessarily be regarded as a stress factor (Bajji et al., 1998).

The present work was therefore undertaken to determine if a coastal population adapted to salt stress and an inland population adapted to non-saline drought conditions display, or not, similar properties in terms of osmotic adjustment. Plants from those populations were exposed to salt stress (NaCl) or osmotic stress [polyethylene glycol (PEG); 15%] for a short (24 h) and a long period (10 d) in a fully controlled environment and then allowed to recover. Water status and ion nutrition of the stressed plants were studied in relation to the synthesis and accumulation of proline, glycinebetaine, and total soluble sugars. Exogenous application of proline and glycinebetaine was also tested to confirm the putative involvement of these compounds in the stress resistance mechanisms of the two contrasting populations.

**Materials and methods**

**Plant material and growth conditions**

Fruits of *A. halimus* L. were collected from wild plants growing at two different sites in Tunisia: Monastir, a coastal site from east Tunisia (36°13′N, 10°23′W), and Sbikha, an arid inland site (36°27′N, 9°49′W). The mean annual pluviometry (average estimated over the previous 3 years) for Monastir and Sbikha was 346 mm and 223 mm, respectively. The mean relative humidity and the mean annual temperature are, respectively, 72.9% and 20.0 °C for Monastir and 50.3% and 25.2 °C for Sbikha. For both sites, an experimental field of ~50 a spontaneously colonized by shrubs of *A. halimus* was considered. The soil salinity level was estimated on 10 independent soil samples per site and quantified according to Kalra and Maynard (1991): mean electrical conductivity (EC) was 2.23 ± 0.43 dS m\(^{-1}\) for Sbikha and 7.18 ± 1.03 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 10 plants at each site and pooled in order to constitute a 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 photosynthetic active radiation (PAR) of 150 \(\mu\)mol 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 2.0 l of nutrient solution containing (in mM) 5 KN0\(_3\), 1 NH\(_4\)H\(_2\)PO\(_4\), 0.5 MgSO\(_4\), and 5.5 Ca(NO\(_3\))\(_2\), and (in µM) 25 KCl, 10 H\(_2\)BO\(_3\), 1 MnSO\(_4\), 1 ZnSO\(_4\), 0.25 CuSO\(_4\), 10 Na\(_2\)MoO\(_4\), and 50 mg l\(^{-1}\) FeEDTA (EC 0.94 dS cm\(^{-1}\), \(\Psi_s = -0.68\) MPa). 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).

Stress treatment was applied 10 d after transfer to nutrient solution. For salt stress, NaCl was added to the nutrient solution to obtain a final concentration of 40 (low salt, EC 5.77 dS cm⁻¹ and \( \Psi_w = -0.25 \) MPa) and 160 mM (high salt, EC 17.4 dS cm⁻¹ and \( \Psi_w = -0.68 \) MPa). For water stress, PEG (10 000; Sigma Aldrich, Belgium) was added in nutrient solution to reach a final dose of 15% (EC 1.02 dS cm⁻¹ and \( \Psi_w = -0.46 \) MPa). Plants maintained in the absence of NaCl and PEG were used as the control. 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. The remaining plants were then transferred back to the control solution for another period of 10 d (recovery period): 12 of them were harvested after 24 h and 12 after 10 d of recovery.

### Influence of exogenous proline and glycinebetaine

In another set of experiments, plants from these populations were exposed under the same environmental conditions to similar stressing factors, as stated above, except that 1 mM proline or 1 mM glycinebetaine was added to the nutrient solution (Sigma Aldrich, Belgium) according to Martínez (2001).

### Plant growth and water status

At the time of harvest, shoot and root fresh weights (FW) were estimated and organs were then immediately incubated in an oven at 70 °C. Root and shoot dry weights (DW) as well as water content were estimated after 48 h. Shoot water potential (\( \Psi_s \)) and \( \Psi_w \) were determined between 12.00 and 14.00. Shoot \( \Psi_w \) was evaluated immediately after sampling using the pressure chamber method. For \( \Psi_s \) determination, tissues were quickly collected, cut into small segments, placed in Eppendorf tubes perforated with four small holes, and immediately frozen in liquid nitrogen. After being encased individually in a second intact Eppendorf tube, they were allowed to thaw for 30 min and centrifuged at 15 000 g for 15 min at 4 °C. The collected tissue sap was analysed for \( \Psi_s \) estimation. Osmolarity (\( \sigma \)) was assessed with a vapour pressure osmometer (Wescor 5500) and converted from mosmol kg⁻¹ to MPa using the formula: \( \Psi_s\) (MPa) = \((\text{mosmol kg}^{-1}) \times 2.58 \times 10^{-3}\) according to the Van’t Hoff equation.

To eliminate the effect of water loss on the possible changes in \( \Psi_s \) (which should not be regarded as osmotic adjustment sensu stricto), \( \Psi_s \) values were adjusted to the water content of unstressed tissues according to \( X \times Y / Z \), where \( X \) is the measured \( \Psi_s \), and \( Y \) and \( Z \) are the water content of the stressed and unstressed tissues, respectively.

### Ion concentration and malondialdehyde (MDA) quantification

For major cations (K⁺, Na⁺, Mg²⁺, and Ca²⁺) and inorganic phosphate quantification, tissues harvested on five plants per treatment were oven-dried at 80 °C for 48 h, and 50 mg DW were digested in 35% (v/v) HNO₃. Analyses were conducted by flame atomic absorption spectrophotometry (VARIAN spectra-300).

MDA, routinely used as an indicator of membrane lipid peroxidation, was extracted from 250 mg of fresh tissue homogenized with 5% (w/v) trichloroacetic acid and determined according to Heath and Packer (1968).

### Analysis of organic solutes

Tissues belonging to six plants per treatment were pooled prior to organic compounds analysis and quickly frozen in liquid nitrogen.

For glycinebetaine determination, collected leaves and roots (~200 mg) were mixed with 5 ml of 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 mm ×4.6 mm) preceded by a precolumn (10 mm ×1 mm) packed with the same phase. The mobile phase contained 13 mM sodium heptane sulphonic acid and 5 mM Na₂SO₄ in deionized water (pH adjusted to 3.7 with 1 N H₂SO₄) at a flow rate of 0.8 ml min⁻¹. Detection was by a UV detector (Bio-Rad 1801 UV monitor) and quantification was performed with the ValueChrom™ HPLC system (BioRad Chromatography Software version 4).

For free proline quantification, 1 g of tissue was extracted with 5 ml of 5% salicylic acid; after centrifugation at 5000 g, free proline was specifically quantified according to Bates et al. (1973). Soluble sugars were extracted in 80% ethanol from 1 g of leaf fresh tissue and quantified by the classical anthrone method (Yemm and Willis, 1954) using a spectrophotometer (Beckman DU™ 640). A standard curve was established using glucose and the results are therefore expressed in μmol equivalent glucose g⁻¹ FW. For each type of compound, extraction was performed on three distinct subsamples and all measurements for a given subsample were performed in triplicate. Concentrations were expressed on the basis of a constant water content applying the same correcting factor used previously for \( \Psi_s \) (see above).

### Photosynthesis-related parameters

Instantaneous CO₂ assimilation in saturating conditions and transpiration rates were measured using a CO₂ and water vapour analyser (LCA 2 8.7; ADC, Herts, UK) and an air supply unit (ASU 10.87; ADC), mounted in series in an open system. Gas exchange was first measured using a PLC (N) Parkinson leaf cuvette on intact leaves for 1 min (20 records min⁻¹), with an air flow of 300 ml min⁻¹. Leaves were illuminated with four Philips mercury-vapour lamps (HPLN 400 W). The photosynthetic photon flux density (PPFD) at the leaf surface was set to 500 μmol m⁻² s⁻¹. To quantify the impact of water stress on the chemical processes of photosynthesis independently of its effect on stomatal closure, a CO₂ molar ratio was used that was high enough to saturate the carboxylation sites. Air jets of 1000 μmol mol⁻¹ CO₂ and 2% O₂ in N₂ were directed to both surfaces; instantaneous CO₂ assimilation in saturating conditions (A) and instantaneous transpiration rate (E) readings were corrected for water vapour, temperature, and atmospheric pressure (von Caemmerer and Farquhar, 1981). Instantaneous water use efficiency (WUEₑ) is given as the ratio of A to E. Leaf stomatal conductance (gₛ) was independently measured on the abaxial surface of leaves with an automatic porometer (MK III; Delta-T Devices, UK). For each treatment, six separated plants were considered.

Chlorophyll fluorescence measurements were carried out using a portable pulse-modulated chlorophyll fluorimeter (FMS2; Hansatech, King’s Lynn, UK). The recordings were performed both on the last fully developed leaf (which appears during stress imposition) and on the third mature leaf (already present at the time of stress imposition). All measurements (10 per treatment) were performed on the middle part of the abaxial side of the leaves. Measurements were also performed at the end of the recovery period on the last fully expanded leaf. Leaf portions were acclimated to darkness for 30 min. The minimal fluorescence level (\( F₀ \)) was measured by measuring the modulated light (0.1 μmol m⁻² s⁻¹). The maximal fluorescence level (\( Fₘ \)) with all photosystem II (PSII) reaction centres closed was determined by a 0.8 s saturating pulse at 8500 μmol m⁻² s⁻¹ in dark-adapted leaves. The
leaf was then continuously illuminated with white actinic light (320 
\mu\text{mol m}^{-2} \text{s}^{-1}). After 7 min, the steady-state value of fluorescence 
\(F_{s}\) was recorded and a second saturating pulse at 8500 \mu\text{mol m}^{-2} 
\text{s}^{-1} was imposed to determine maximal fluorescence level in the light-adapted state 
\(F_{m}'\). The actinic light was removed and the minimal fluorescence level in the light-adapted state 
\(F_{0}'\) was determined by illuminating the leaf with a 3 s pulse of far-red. Using both light and dark fluorescence parameters, the following 
were calculated: the maximal efficiency of PSII photochemistry in the dark-adapted state, 
\(\Phi_{\text{PSII}} = (F_{m}' - F_{0}')/(F_{m}' - F_{0})\); the efficiency of excitation capture by open PSII reaction centres, 
\(F_{v}/F_{m}' = (F_{m}' - F_{0}')/F_{m}'\); the non-photochemical quenching NPQ = \((F_{m}' - F_{m})/F_{m}'\); and the actual PSII efficiency, 
\(\Phi_{\text{PSII}} = (F_{m}' - F_{0}')/F_{m}'\).

Chlorophyll (Chl \(a\) and Chl \(b\)) and total carotenoid (xanthophylls+\(\beta\)-carotene) concentrations were determined using 
100 mg FW of leaf material ground in a pre-chilled mortar in the presence of 8 ml of acetone 80% (v/v). After complete extraction, 
the mixture was filtered and the volume adjusted to 10 ml with cold acetone. The absorbance of the extract was read at 663.2, 646.8, and 
470 nm using a Beckman DU640 spectrophotometer, and pigment 
concentrations were calculated according to Lichtenthaler (1987).

**Statistical treatment of the data**

Three independent experiments were performed with results 
exhibiting similar tendencies. Data were analysed using a two-way 
ANOVA (analysis of variance) at a significance level of 
\(P = 0.05\) (*) or \(P < 0.01\) (**). The model is defined on the basis of fixed 
main effects (treatment and population) considering separately the 
two periods of stress exposure. When the ANOVA was significant 
at \(P = 0.05\), the Duncan multiple range test was used for mean 
comparison. Data were analysed using the SAS software (SAS 
Institutes, Tervueren, Belgium).

**Results**

Plants from Sbikha and from Monastir exhibited a similar 
dry weight under control conditions as shown in Fig. 1 for 
shoots after 10 d of treatment. A moderate dose of NaCl 
(40 mM) improved plant growth after 10 d of treatment in 
plants from both populations. The highest dose of salt 
(160 mM NaCl) reduced the root (detailed data not 
shown) and the shoot (Fig. 1) dry weight in plants from 
Sbikha but not in those originating from Monastir. 
Conversely, the impact of 15% PEG was more deleterious 
in plants from the coastal area Monastir than in those from 
Sbikha. Shoot growth was able to resume fully during the 
recovery period for plants previously exposed to 15% 
PEG, while those from Sbikha and previously exposed to 
160 mM NaCl only partly recovered (Fig. 1). 

Shoot \(\Psi_{w}\) was already slightly reduced after 24 h in 
response to 160 mM NaCl and to a higher extent in plants 
from Monastir than in those from Sbikha (Table 1). After 
10 d of treatment, salt-treated plants from both populations 
exhibited a similar \(\Psi_{w}\) value in response to salt 
treatments. During the stress period, shoot \(\Psi_{w}\) remained 
lower in plants exposed to PEG than in those exposed to 
the highest dose of NaCl and was lower in Sbikha than in 
Monastir plants. Shoot \(\Psi_{w}\) had already increased after 
24 h of recovery and both populations presented similar 
\(\Psi_{w}\) values, whatever the preceding stressing treatment 
(Table 1). The leaf \(\Psi_{s}\) decreased in response to 40 mM 
NaCl after 24 h in Sbikha plants and after 10 d in both 
populations. A salt-induced decrease in \(\Psi_{s}\) occurred more 
quickly and was more pronounced in Monastir than in 
Sbikha plants. An inverse trend was recorded for PEG-
treated plants where seedlings from Sbikha exhibited the 
strongest decrease in \(\Psi_{s}\) after only 24 h of exposure. In 
contrast to \(\Psi_{s}\), osmotic potential still remained low 24 h 
after transfer to control solution, especially in seedlings 
from Monastir. At the end of the recovery period, a lower 
\(\Psi_{s}\) than in non-stressed controls was still recorded for 
Monastir plants previously exposed to the highest dose of 
salt or to 15% PEG.

The root and shoot Na\(^{+}\) concentrations (Fig. 2) were 
always lower in Sbikha than in Monastir plants, whatever 
the external dose of NaCl or the duration of exposure, 
thus suggesting that the higher salinity resistance of the 
latter could not be attributed to the restriction of sodium 
absorption and accumulation. Salt stress slightly decreased 
the shoot K\(^{+}\) concentration (detailed data not shown), 
although such a decrease was significant for Sbikha plants 
exposed for 10 d to 160 mM NaCl only (from 1.15 mmol 
g\(^{-1}\) DW in control to 0.78 mmol g\(^{-1}\) DW in stressed 
plants; \(P < 0.01\)). By contrast, salt and water stress had no 
impact on Mg\(^{2+}\), Ca\(^{2+}\), or P concentrations.

Proline accumulation occurred within 24 h following 
stress imposition (Fig. 3). For a given population, proline
Table 1. Shoot water potential \((\Psi_w)\) and leaf osmotic potential \((\Psi_s)\) in seedlings of Atriplex halimus from a coastal site (Monastir) and an inland area (Sbikha) and exposed for 1 d or 10 d to the control nutrient solution or to a the nutrient solution containing 40 mM or 160 mM NaCl or 15% PEG (stress period)

Seedlings previously exposed to stress were then transferred to the control solution and allowed to recover for 1 d or 10 d (recovery period). Each value is the mean of six replicates ± standard error of the mean. For a given parameter and a given time, values sharing a common letter are not significantly different at \(P < 0.05\).

<table>
<thead>
<tr>
<th>Water potential ((\Psi_w)) in MPa</th>
<th>Population</th>
<th>Stress period</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 d</td>
<td>10 d</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monastir</td>
<td>–0.63 ± 0.04 a</td>
<td>–0.72 ± 0.02 a</td>
<td>–0.58 ± 0.03 a</td>
</tr>
<tr>
<td>Sbikha</td>
<td>–0.68 ± 0.03 a</td>
<td>–0.69 ± 0.05 a</td>
<td>–0.62 ± 0.04 a</td>
</tr>
<tr>
<td>40 mM NaCl</td>
<td>Monastir</td>
<td>–0.71 ± 0.03 a</td>
<td>–0.94 ± 0.03 b</td>
</tr>
<tr>
<td>Sbikha</td>
<td>–0.77 ± 0.02 b</td>
<td>–0.92 ± 0.01 b</td>
<td>–0.73 ± 0.08 a</td>
</tr>
<tr>
<td>160 mM NaCl</td>
<td>Monastir</td>
<td>–1.33 ± 0.05 d</td>
<td>–1.42 ± 0.04 d</td>
</tr>
<tr>
<td>Sbikha</td>
<td>–1.19 ± 0.04 c</td>
<td>–1.27 ± 0.04 c</td>
<td>–0.59 ± 0.06 a</td>
</tr>
<tr>
<td>15% PEG</td>
<td>Monastir</td>
<td>–1.54 ± 0.01 e</td>
<td>–1.68 ± 0.02 e</td>
</tr>
<tr>
<td>Sbikha</td>
<td>–1.74 ± 0.07 f</td>
<td>–1.95 ± 0.01 f</td>
<td>–0.69 ± 0.05 a</td>
</tr>
<tr>
<td>Osmotic potential ((\Psi_s)) in MPa</td>
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<tr>
<td>Control</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Monastir</td>
<td>–1.69 ± 0.07 a</td>
<td>–1.54 ± 0.05 a</td>
<td>–1.48 ± 0.01 a</td>
</tr>
<tr>
<td>Sbikha</td>
<td>–1.72 ± 0.07 a</td>
<td>–1.63 ± 0.04 a</td>
<td>–1.53 ± 0.04 a</td>
</tr>
<tr>
<td>40 mM NaCl</td>
<td>Monastir</td>
<td>–1.78 ± 0.11 a</td>
<td>–2.34 ± 0.03 b</td>
</tr>
<tr>
<td>Sbikha</td>
<td>–2.01 ± 0.04 b</td>
<td>–2.26 ± 0.05 b</td>
<td>–1.63 ± 0.06 ab</td>
</tr>
<tr>
<td>160 mM NaCl</td>
<td>Monastir</td>
<td>–2.33 ± 0.09 c</td>
<td>–2.74 ± 0.10 c</td>
</tr>
<tr>
<td>Sbikha</td>
<td>–2.09 ± 0.03 b</td>
<td>–2.23 ± 0.11 b</td>
<td>–1.88 ± 0.16 b</td>
</tr>
<tr>
<td>15% PEG</td>
<td>Monastir</td>
<td>–2.56 ± 0.18 c</td>
<td>–2.83 ± 0.07 c</td>
</tr>
<tr>
<td>Sbikha</td>
<td>–3.13 ± 0.12 d</td>
<td>–3.67 ± 0.06 d</td>
<td>–1.98 ± 0.09 b</td>
</tr>
</tbody>
</table>

Accumulation was higher in response to water stress than in response to salt stress. Whatever the stressing agent, shoot proline concentration was higher in Sbikha than in Monastir plants \((P < 0.01)\). Proline concentration quickly decreased after the stress relief (although at a higher rate for plants previously exposed to PEG than for plants exposed to NaCl). The accumulation of glycinebetaine required a longer duration of treatment since it occurred between day 1 and day 10. The concentration of glycinebetaine was higher in Monastir than in Sbikha and was always higher in salt-treated plants compared with those exposed to osmotic stress. In contrast to proline, the glycinebetaine concentration only marginally decreased after the stress relief. Total soluble sugars in control plants was the same for the two considered populations (overall mean of 55.2 μmol equivalent glucose g⁻¹ FW) and increased in a similar way in response to salt stress for both populations (45% and 69% of increase in response to 40 mM and 160 mM NaCl, respectively). Total soluble sugars also increased in response to PEG, but to a greater extent in Sbikha (94% of increase) than in Monastir (57%) plants.

Instantaneous CO₂ assimilation rate \((A)\) remained unaffected by 24 h of exposure to salt stress and was only slightly reduced by 15% PEG (detailed data not shown). After 10 d of treatment, \(A\) was still similar in plants exposed to 40 mM NaCl and in controls (Table 2). A high dose of salt (160 mM NaCl) reduced the value of \(A\) in both populations but to a higher extent in Sbikha than in Monastir, while an inverse trend was recorded in response to osmotic stress induced by PEG. Plants from Sbikha always displayed a lower stomatal conductance than plants from Monastir \((P < 0.05)\), except under control conditions (Table 2). Significant differences between populations were also recorded for WUEᵢ (Table 2): the moderate NaCl dose (40 mM NaCl) had no impact on the WUEᵢ of Monastir plants but it significantly increased the WUEᵢ for plants from Sbikha. Both populations exhibited similar WUEᵢ in response to 160 mM NaCl despite the fact that the \(A\) values were clearly higher in Monastir than in Sbikha. In the presence of 15% PEG, WUEᵢ was twice as high for the latter as for the former.

Generation of reactive oxygen species is usually enhanced under environmental stress and is commonly quantified on the basis of MDA concentration. As indicated in Fig. 4, MDA concentration remained constant under control conditions and in response to 40 mM NaCl. The highest stress intensities (160 mM NaCl and 15% PEG) strongly increased the MDA concentration but to a higher extent in Monastir than in Sbikha plants after both 1 d and 10 d of treatment. Considering only the stressed plants, a significant negative correlation was found between proline concentration and stomatal conductance \((r^2=0.89; P < 0.01)\) on the one hand, as well as between proline and MDA concentration \((r^2=0.74; P < 0.05)\) on the other hand. By contrast, there was no correlation between glycinebetaine concentration and stomatal conductance or MDA concentration.
Chlorophyll concentrations remained unaffected, whatever the population or the treatment (mean values of 1.25 mg g\(^{-1}\) FW and 0.42 mg g\(^{-1}\) FW for Chl \(a\) and Chl \(b\), respectively). Carotenoid concentration (mean values of 0.25 mg g\(^{-1}\) FW for controls) was reduced in response to water stress and to a higher extent in Sbikha (decrease of 18.3%; \(P < 0.01\)) than in Monastir (decrease of 8.7%; \(P < 0.05\)) plants. Most chlorophyll fluorescence-related parameters remained unaffected after 24 h of exposure to stress, except \(F_v/F_m\) which significantly decreased by 8% and 12% in seedlings from Sbikha exposed to 160 mM NaCl and 15% PEG, respectively. After 10 d of exposure (Table 3), no significant difference was recorded between the two populations maintained under control conditions or low NaCl dose (except a slight decrease of \(F_v/F_m\) in Sbikha exposed to 40 mM NaCl). Higher stress intensities (160 mM NaCl and 15% PEG) induced a decrease in \(F_v/F_m\), \(q_P\), \(F_v'/F_m'\), and \(\Phi_{PSII}\), as well as an increase in NPQ, and those modifications were always more important in Sbikha than in Monastir plants. As far as stressed plants were concerned, there was a significant positive correlation (\(r^2=0.73\); \(P < 0.01\)) between glycinebetaine concentration and \(\Phi_{PSII}\) but not between \(\Psi_{PSII}\) and proline content.

Taken together, the present data suggest that proline is mainly produced in response to water stress by individuals adapted to drought conditions and that, beside osmotic adjustment, it may act through the protection of plant tissues against oxidative stress. By contrast, glycinebetaine appeared to be produced more efficiently by coastal halophyte populations exposed to NaCl and acts through
The maintenance of PSII integrity. Thus both populations were tentatively exposed to the considered stress in the presence of 1 mM proline or 1 mM glycinebetaine added to the nutrient solution. Neither proline nor glycinebetaine had any deleterious impact on plant growth or behaviour for the considered duration of the experiment. As indicated in Table 4, both exogenous proline and glycinebetaine were efficiently absorbed by roots of non-stressed plants and accumulated within shoots to a similar extent in the two considered populations of *A. halimus*. Both populations simultaneously treated with proline and 160 mM NaCl or with proline and 15% PEG accumulated similar amounts of proline at the shoot level. A similar observation was valid for glycinebetaine concentration in the case of plants maintained in the presence of this compound in the nutrient solution (Table 4), thus suggesting that absorption of these compounds and endogenous synthesis are not fully additive and that accumulation resulting from absorption might somewhat reduce, probably through feedback inhibition, the synthesis of the corresponding endogenous compound. Exogenous glycinebetaine had no impact on endogenous proline concentration and, similarly, exogenous proline had no effect on endogenous glycinebetaine content (data not shown). Under the present experimental conditions, neither proline nor glycinebetaine had any impact on endogenous Na⁺ and K⁺ concentrations in the shoots of stressed plants (data not shown).

Exogenous proline had no impact on the growth of plants from Sbikha and exposed to PEG or NaCl, but it clearly mitigated the deleterious impact of PEG on shoot growth of plants from Monastir (Fig. 5). In this latter case, exogenous proline clearly increased A values and restricted stomatal conductance (Fig. 5). Another obvious effect of exogenous proline was to reduce significantly MDA content in all stressed shoot tissues, except in salt-treated Sbikha plants (Table 5).

Exogenous glycinebetaine did not improve the behaviour of PEG-treated plants and had no impact on Monastir exposed to NaCl. By contrast, it clearly improved shoot growth of Sbikha plants exposed to NaCl in relation to an improvement of \( \Phi_{\text{PSII}} \) and net assimilation rate (Fig. 5). Glycinebetaine also increased stomatal conductance in NaCl-treated plants from Sbikha. However, in contrast to proline, exogenous glycinebetaine had no impact on MDA concentration, whatever the treatment or the population considered (Table 5). A greater decrease in \( \Psi_s \) value in response to stress was recorded in Sbikha plants exposed to NaCl in the presence of exogenous glycinebetaine and in Monastir plants exposed to PEG in the presence of exogenous proline; in both cases, this effect was related to an increase in the shoot total soluble sugar concentration (Table 5).

**Discussion**

It has been frequently reported that glycinebetaine acts as the main stress-induced agent involved in the osmotic adjustment and protection of cellular structures in plant species belonging to the Chenopodiaceae family (Rhodes and Hanson, 1993; Hanson *et al.*, 1994). The present work demonstrates that a given xero-halophyte plant

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**Table 2. Instantaneous CO₂ assimilation rate (A, \( \mu \text{mol CO}_2 \ \text{m}^{-2} \ \text{s}^{-1} \)), stomatal conductance (gₛ, cm s⁻¹), and instantaneous water-use efficiency (WUEᵢ) in seedlings of *Atriplex halimus* from a coastal site (Monastir) and an inland area (Sbikha) and exposed for 10 d to the control nutrient solution or to the nutrient solution containing 40 mM or 160 mM NaCl or 15% PEG (stress period)

<table>
<thead>
<tr>
<th>Population</th>
<th>NaCl 40 mM</th>
<th>NaCl 160 mM</th>
<th>PEG 15%</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monastir</td>
<td>36.4 ± 1.8 a</td>
<td>32.5 ± 2.4 ab</td>
<td>28.4 ± 0.9 b</td>
<td>39.8 ± 1.7 a</td>
</tr>
<tr>
<td>Sbikha</td>
<td>39.8 ± 1.7 a</td>
<td>40.3 ± 2.1 a</td>
<td>16.9 ± 1.4 c</td>
<td>35.8 ± 1.7 a</td>
</tr>
</tbody>
</table>

Each value is the mean of six replicates ± standard error of the mean. For a given parameter and a given time, values sharing a common letter are not significantly different at \( P < 0.05 \).
species may simultaneously use different organic solutes for osmoregulation and protection of cellular structures but that the relative importance and kinetics of accumulation of each of them may be, at least partly, related to the environmental characteristics of the habitat occupied by the population considered. Indeed, the present results suggest that, as far as *A. halimus* is concerned, glycinebetaine is preferentially accumulated by coastal populations adapted to saline areas while proline accumulates mainly in response to osmotic stress and to a higher extent in an inland population growing under non-saline conditions. These observations are reinforced by the fact that exogenous proline improved the response of the coastal population to water-stress conditions, while exogenous glycinebetaine improved the behaviour of the inland population exposed to high doses of salt (Fig. 5).

It has to be noted that proline and glycinebetaine concentration expressed at the whole tissue level remain in the same range. This, however, does not imply that they assume similar functions in terms of osmotic adjustment of specific cell compartments. It has been demonstrated that glycinebetaine accumulates mainly in the chloroplasts while proline accumulates in the cytosol (McNeil et al., 1999). In this respect, accumulation of the two solutes considered may appear complementary. Beside proline and glycinebetaine, soluble sugars also accumulate in response to both salinity and water stress. According to Martínez et al. (2005) soluble sugars may even be considered as the main contributors to osmotic adjustment in *A. halimus*. Soluble sugars accumulated in response to 40 mM NaCl while neither growth (Fig. 1) nor CO₂ assimilation rates (Table 2) were affected. Moreover, a strong positive correlation was found between sugar accumulation in response to exogenous glycinebetaine application and internal $\Psi_h$. Thus, the present data confirm that, beside proline and glycinebetaine, sugars may be involved in osmotic adjustment. Ions may also contribute to osmotic adjustment, especially in response to salt-stress conditions (Cerda et al., 1995). The effective contribution of Na⁺ to osmotic adjustment is nevertheless difficult to quantify in the case of *A. halimus* since a consistent portion of Na⁺ may be excreted to the trichomes present at high densities at the leaf surface; although Na⁺ concentration had already increased after 24 h of exposure to NaCl (Fig. 4), there is no estimation of the proportion which was removed from the mesophyll to the leaf surface. In a recent study, LeFèvre (2007) demonstrated that >50% of the accumulated Na⁺ may be excreted at the leaf surface, but this percentage may vary according to both the age of the leaf and the environmental conditions. Moreover, efficient osmotic adjustment was observed in response to 15% PEG (Table 1) while no modification was recorded in the mineral content for plants exposed to this treatment. As far as PEG-treated plants were concerned, differences

### Table 3. Maximal efficiency of PSII photochemistry in the dark-adapted state ($F_v/F_m$), the photochemical quenching coefficient ($q_P$), the efficiency of excitation capture by open PSII reaction centres ($F_v'/F_m'$), the non-photochemical quenching (NPQ), and the actual PSII efficiency ($\Phi_{PSII}$) in seedlings of *Atriplex halimus* from a coastal site (Monastir) and an inland area (Sbikha) and exposed for 10 d to the control nutrient solution or to a the nutrient solution containing 40 mM or 160 mM NaCl or 15% PEG

Each value is the mean of 10 replicates ± standard error of the mean. For a given parameter and a given time, values sharing a common letter are not significantly different at $P<0.05$.

<table>
<thead>
<tr>
<th>Population</th>
<th>$F_v/F_m$</th>
<th>$q_P$</th>
<th>$F_v'/F_m'$</th>
<th>NPQ</th>
<th>$\Phi_{PSII}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.83±0.04 a</td>
<td>0.62±0.02 a</td>
<td>0.59±0.03 a</td>
<td>0.75±0.05 a</td>
<td>0.37±0.05 a</td>
</tr>
<tr>
<td>Sbikha</td>
<td>0.80±0.03 a</td>
<td>0.61±0.03 a</td>
<td>0.63±0.01 a</td>
<td>0.68±0.04 a</td>
<td>0.38±0.01 a</td>
</tr>
<tr>
<td>40 mM NaCl</td>
<td>0.79±0.02 a</td>
<td>0.58±0.03 a</td>
<td>0.62±0.04 a</td>
<td>0.73±0.02 a</td>
<td>0.36±0.04 a</td>
</tr>
<tr>
<td>Sbikha</td>
<td>0.75±0.02 b</td>
<td>0.62±0.02 a</td>
<td>0.63±0.01 a</td>
<td>0.76±0.03 a</td>
<td>0.39±0.02 a</td>
</tr>
<tr>
<td>160 mM NaCl</td>
<td>0.78±0.02 ab</td>
<td>0.53±0.02 b</td>
<td>0.50±0.02 b</td>
<td>1.02±0.04 b</td>
<td>0.27±0.04 b</td>
</tr>
<tr>
<td>Sbikha</td>
<td>0.62±0.03 c</td>
<td>0.31±0.04 d</td>
<td>0.32±0.00 d</td>
<td>1.87±0.05 d</td>
<td>0.10±0.02 c</td>
</tr>
<tr>
<td>15% PEG</td>
<td>0.72±0.02 b</td>
<td>0.49±0.03 b</td>
<td>0.52±0.03 b</td>
<td>1.23±0.01 c</td>
<td>0.25±0.03 b</td>
</tr>
<tr>
<td>Sbikha</td>
<td>0.60±0.01 c</td>
<td>0.37±0.01 c</td>
<td>0.41±0.01 c</td>
<td>2.04±0.02 e</td>
<td>0.15±0.01 c</td>
</tr>
</tbody>
</table>

### Table 4. Shoot proline and glycinebetaine concentration (µmol g⁻¹ FW) in seedlings of *Atriplex halimus* from a coastal site (Monastir) and an inland area (Sbikha) and exposed for 10 d to the nutrient solution containing 1 mM proline (Pro) or 1 mM glycinebetaine (GB) in the presence or absence of 160 mM NaCl or 15% PEG

Each value is the mean of five replicates ± standard errors. For a given organic solute, means followed by the same letter are not significantly different at $P=0.05$.

<table>
<thead>
<tr>
<th>Quantified compound</th>
<th>Type of solution</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Monastir</td>
</tr>
<tr>
<td>Proline</td>
<td>Control</td>
<td>19.3±1.2 a</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>36.5±1.8 b</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>40.3±0.5 bc</td>
</tr>
<tr>
<td></td>
<td>Pro</td>
<td>68.3±3.8 e</td>
</tr>
<tr>
<td></td>
<td>Pro+NaCl</td>
<td>71.3±5.8 e</td>
</tr>
<tr>
<td></td>
<td>Pro+PEG</td>
<td>77.9±2.9 f</td>
</tr>
<tr>
<td>Glycinebetaine</td>
<td>Control</td>
<td>3.4±0.2 a</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>27.6±1.4 cd</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>54.4±0.9 c</td>
</tr>
<tr>
<td></td>
<td>GB</td>
<td>30.4±2.5 d</td>
</tr>
<tr>
<td></td>
<td>GB+NaCl</td>
<td>33.6±3.4 d</td>
</tr>
<tr>
<td></td>
<td>GB+PEG</td>
<td>32.2±2.9 d</td>
</tr>
</tbody>
</table>
between Sbikha and Monastir plants were higher for $\Psi_s$ than for $\Psi_w$ (Table 1), which suggests that the maintenance of turgor was more efficient in the former than in the latter. This could at least partly explain the higher shoot dry weight recorded for Sbikha plants after 10 d of exposure (Fig. 1).

The present data demonstrate that under both salt- and drought-stress conditions, proline accumulated more rapidly than glycinebetaine. It could therefore be inferred that proline is involved in the plant responses during the first hour of stress while glycinebetaine accumulated if the environmental constraint persists. According to Munns (2002), the first impact on plants exposed to salinity is caused by the osmotic component of salt stress, while ionspecific effects linked to the rise of endogenous Na$^+$ and Cl$^-$ take time to develop and only occur in a second step. This is not in contradiction to the fact that in the stressed plant material used in the present study, proline accumulated to higher levels in response to osmotic stress than in response to salinity and to a higher extent in the inland population. It has, however, to be mentioned that for both populations tested a significant rise in Na$^+$ concentration was already recorded after 24 h, even at the lowest dose of NaCl, thus suggesting that the synthesis of glycinebetaine itself required a longer delay than the synthesis of proline, independently of the presence of endogenous Na$^+$ eliciting the response. Niu et al. (2007) and Luo et al. (2007) recently reported that unusual post-transcriptional processing of betaine aldehyde dehydrogenase and choline monooxygenase loci may explain the low level of glycinebetaine registered in some cereal species. It could therefore be of special interest to determine if such a process could be involved in the regulation of glycinebetaine synthesis in plant species belonging to the Chenopodiaceae family. It may also be postulated that other intermediates, such as abscisic acid, could be produced in response to stress in order to induce the expression of genes coding for enzymes involved in

![Fig. 5. Impact of exogenous proline (1 mM) or glycinebetaine (1 mM) on the shoot dry weight, net assimilation rate of CO$_2$ ($A$), stomatal conductance ($g_s$), WUE$_i$, and actual efficiency of PSII ($\Phi_{PSII}$) of Atriplex halimus from a coastal saline site (Monastir) and an inland semi-arid area (Sbikha) and exposed for 10 d to the nutrient solution containing 160 mM NaCl or 15% PEG. For each parameter, values are expressed as a percentage of values recorded for non-stressed seedlings (maintained under the control solution without NaCl, PEG, proline, or glycinebetaine). For a given parameter, asterisks indicate that value differs for proline-treated (hatched columns) or glycinebetaine-treated (black columns) plants in comparison with plants maintained under the same stress conditions in the absence of proline and glycinebetaine (white columns).](https://academic.oup.com/jxb/article-abstract/59/6/1315/484105)
glycinebetaine synthesis, as demonstrated by Ishitani et al. (1995) for barley. Wang and Showalter (2004), however, reported that salt induction of glycinebetaine occurs in an abscisic acid-independent way in *Atriplex prostrata*.

Accumulated proline is rapidly degraded after the stress relief, while glycinebetaine was still present at high concentrations in tissues 10 d after the stress relief, thus corroborating the view that this quaternary ammonium compound is stable and hardly metabolized in plant tissues (Mäkelä et al., 1996). Glycinebetaine may thus constitute a convenient solute for environments characterized by permanent stress, as is the case in Monastir, where soil salinity estimated through EC only slightly varies during the year (A. Ben Hassine, unpublished results). By contrast, although the site of Sbikha is characterized by long periods of drought, erratic precipitation may occur, leading to the relief of the main environmental constraint, and the remobilization of stored proline may therefore be of special interest to sustain growth when water is available.

Beside osmotic adjustment, proline and glycinebetaine are reported to be involved in the protection of cellular structures, but the present data suggest that these osmoprotecting compounds could be involved in different protecting mechanisms. Indeed, glycinebetaine appears to be directly involved in the protection of chloroplast structures, as suggested by the higher $\Phi_{PSII}$ and lower NPQ values recorded for stressed tissues of plants from Monastir compared with those from Sbikha (Table 3), as well as the positive effect of exojogenic glycinebetaine on $\Phi_{PSII}$ of salt-treated plants from Sbikha (Fig. 5). It is well known that salinity induces a wide range of perturbations in terms of chlorophyll concentration and chloroplast structure (Abdelkader et al., 2007). The quantum yield of PSII photochemistry ($F_v/F_m$) declined to a greater extent in plants from Sbikha than in those from Monastir, and a similar trend was recorded for $\Phi_{PSII}$ (Table 3). Surprisingly, exogenous glycinebetaine improved the performance and stability of PSII only in response to salt stress but not in response to PEG and only in the population which exhibited the lowest capacity to synthesize this quaternary ammonium compound. These data suggest that the impact of salinity, on the one hand, and osmotic stress, on the other hand, may differ even if the ultimate consequence of both stresses is a change in similar fluorescence-related parameters. It may be hypothesized that a significant portion of the Na$^+$ and Cl$^-$ absorbed could accumulate within the chloroplast and directly interact with thylakoid membranes, although precise data in the literature are still crucially lacking in this respect. PSII integrity could be related to antioxidative systems. Nevertheless, glycinebetaine is an amphiphilic zwitterionic fully N-methyl-substituted derivative of glycine which could directly interact with accumulated ions or protect membranes from the deleterious effect of an excess of charges in the surrounding medium independently of oxidative damage. Some authors, however, assume that glycinebetaine is not directly involved in the immediate protection of thylakoid structures but rather accelerates the repair of salt-induced damage (Ohnishi and Murata, 2006; Yang et al., 2007).

The present data also suggest that exogenous glycinebetaine added to the nutrient solution is absorbed by the roots, efficiently translocated to the shoots, and correctly targeted to the chloroplasts. Génard et al. (1991) demonstrated that glycinebetaine may cross the chloroplast envelopes *in vitro*, and Schwacke et al. (1999) identified a non-specific glycinebetaine transporter exhibiting a low $K_m$ for this quaternary ammonium compound. Nevertheless, most if not all glycinebetaine synthesis is thought to occur in chloroplasts from imported choline (Nuccio et al., 2000) and data concerning glycinebetaine transfer to and from the chloroplast compartment are not yet available.

Glycinebetaine has also been reported to protect cellular structures against the deleterious impact of oxidative stress. Yang et al. (2007) reported that genetically engineered tobacco with the ability to accumulate glycinebetaine showed a higher content of ascorbate and reduced glutathione as well as an increase in the activity of superoxide dismutase. Hoque et al. (2007) reported that both proline and glycinebetaine may alleviate the deleterious impact of salt stress on catalase and ascorbate peroxidase activities but that proline is more effective than glycinebetaine. Similarly, Okuma et al. (2004)

### Table 5. Impact of exogenous proline (Pro; 1 mM) or glycinebetaine (GB; 1 mM) on the shoot malondialdehyde concentration (MDA), osmotic potential ($\Psi_s$), and leaf total soluble sugar (TSS) concentrations of *Atriplex halimus* from a coastal saline site (Monastir) and an inland arid area (Sbikha) and exposed for 10 d to the nutrient solution containing 160 mM NaCl or 15% PEG.

<table>
<thead>
<tr>
<th>Population</th>
<th>Treatment</th>
<th>MDA</th>
<th>$\Psi_s$</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sbikha</td>
<td>NaCl</td>
<td>123.9</td>
<td>121.5</td>
<td>153.1</td>
</tr>
<tr>
<td></td>
<td>NaCl+Pro</td>
<td>126.3</td>
<td>119.6</td>
<td>147.6</td>
</tr>
<tr>
<td></td>
<td>NaCl+GB</td>
<td>132.7</td>
<td>144.2*</td>
<td>229.6*</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>137.7</td>
<td>181.9</td>
<td>188.4</td>
</tr>
<tr>
<td></td>
<td>PEG+Pro</td>
<td>107.8*</td>
<td>207.3</td>
<td>191.2</td>
</tr>
<tr>
<td></td>
<td>PEG+GB</td>
<td>128.9</td>
<td>195.6</td>
<td>173.5</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>214.7</td>
<td>177.9</td>
<td>165.9</td>
</tr>
<tr>
<td></td>
<td>NaCl+Pro</td>
<td>158.3*</td>
<td>154.2</td>
<td>172.5</td>
</tr>
<tr>
<td></td>
<td>NaCl+GB</td>
<td>199.6</td>
<td>164.8</td>
<td>170.3</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>230.1</td>
<td>173.6</td>
<td>157.3</td>
</tr>
<tr>
<td></td>
<td>PEG+Pro</td>
<td>142.8*</td>
<td>182.2</td>
<td>149.9</td>
</tr>
<tr>
<td></td>
<td>PEG+GB</td>
<td>202.7</td>
<td>230.2*</td>
<td>248.1*</td>
</tr>
</tbody>
</table>

For each parameter, values are expressed as a percentage of values recorded for non-stressed seedlings (maintained under the control solution without NaCl, PEG, proline, or glycinebetaine). For a given parameter, asterisks indicate that the value differs for proline or glycinebetaine-treated plants in comparison with plants maintained under the same stress conditions in the absence of proline and glycinebetaine.
reported that proline but not glycinebetaine may act as a free radical scavenger. The present results support this latter view; for a given duration of stress and whatever the nature of the considered environmental constraint, oxidative damage quantified on the basis of MDA concentration was always lower in plants from Sbikha which accumulated a higher concentration of endogenous proline (Fig. 4). Similarly, exogenous application of proline to stressed plants significantly contributed to reduce the increase in MDA concentration (Table 5). The protective role of proline could be mediated by the maintenance of NAD(P)⁺/NAD(P)H ratios at values compatible with metabolism under normal conditions. Proline is considered to play an important role in defence mechanisms of stressed cells, which can ameliorate shifts in redox potential by replenishment of NADP⁺ supply as well. Proline degradation can provide carbon, nitrogen, and an energy source after stress (Hare et al., 1999).

Hanson et al. (1994) had already reported that different plant species adapted to different types of habitat may use different osmocompatible solutes affording selective advantages in particular stress environments. The present results suggest that even within a given xero-halophyte plant species such as A. halimus, plants may use a set of osmocompatible solutes to perform osmotic adjustment under stress conditions but that the proportion of each compound may vary according to the considered population and its natural surrounding environment. Plants adapted to saline soils accumulate glycinebetaine preferentially which helps in the protection of photosynthetic machinery, while plants adapted to an arid but non-saline environment accumulate proline preferentially which may also act in the protection against oxidative stress. The species A. halimus exhibits a high level of phenotypic and genetic variability, both within and among populations (Ortíz-Dorda et al., 2005; Walker et al., 2005). The present study considered only two populations and should thus be extended in the future to a larger set of populations from several contrasting habitats, including cold areas where plants are able to cope with low temperatures.

References


