Is osmotic adjustment required for water stress resistance in the Mediterranean shrub *Atriplex halimus* L?

Juan-Pablo Martínez¹, Stanley Lutts², André Schanck², Mohammed Bajji¹, Jean-Marie Kinet¹

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**Summary**

The effect of water stress was investigated in plants from two populations of *Atriplex halimus* L: Tensift issued from a salt-affected coastal area and Kairouan, originating from an inland dried site. Water deficit was applied by withholding water for 22 days. Shoot dry weight (shoot DW), leaf relative water content (RWC), turgid weight to dry weight ratio (TW/DW), osmotic potential ($\psi_s$), osmotic adjustment (OA), proline, glycinebetaine, and sugar content were determined 1, 8, 15 and 22 days after withholding watering. Water stress induced a decrease in shoot DW, RWC, $\psi_s$, and TW/DW, but an increase in glycinebetaine and sugar leaf contents. The decrease of $\psi_s$ and TW/DW was more marked in Kairouan than in Tensift. At the end of the stress period, Kairouan showed a greater OA compared with Tensift. However, the contribution of net solute accumulation (OA$^{acc}$) was similar in both populations in response to stress. Water stress resistance could thus not be associated with higher OA, although the ability of plants to regulate these metabolic and physiological functions could play an important role under harmful conditions. The possible roles of osmolyte accumulations are discussed in relation to the specific physiological strategy of water-stress-resistance in this species.

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**Keywords**

*Atriplex halimus*; Drought resistance; Glycinebetaine; Osmotic adjustment; Water stress

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**Abbreviations:** DW, dry weight; FW, fresh weight; OA, osmotic adjustment; OA$^{tot}$, total osmotic adjustment; OA$^{acc}$, osmotic adjustment due to the contribution of net solute accumulation; OA$^{conc}$, osmotic adjustment due to solute concentration resulting from changes in non-osmotic volume; $\psi_s$, osmotic potential; RWC, relative water content; TW, turgid weight

*Corresponding author. Tel.: +32-10-47-34-36; fax: +32-10-47-34-35.
E-mail address: lutts@bota.ucl.ac.be (S. Lutts).

¹Present address: Departamento de Producción Agrícola, Laboratorio de Bioquímica, Facultad de Ciencias Agronómicas, Casilla 1004, Chile.

Introduction

Water stress is an increasingly expensive problem for plant production. It is, however, well established that non-cultivated plants growing naturally in semi-desertic areas have evolved many adaptations to counteract water deficit stress. The maintenance of turgor during changes in plant water status may preserve the metabolic processes of the plant and contribute to growth. Osmotic adjustment (OA), defined as lowering of the osmotic potential due to net solute accumulation in response to water stress, may help to achieve this goal and is considered to be a beneficial drought tolerance mechanism in several plant species (Blum, 1989; Zhang et al., 1999; Chimenti et al., 2002; Wang et al., 2003).

The accumulation of compatible solutes in plants has drawn much attention during the last years (Ingram and Bartels, 1996; Bohnert and Jensen, 1996; Hare et al., 1998). It has been hypothesized that these compounds benefit stressed cells in two ways: (i) by acting as cytoplasmic osmolytes, thereby facilitating water uptake and retention (Hare et al., 1998), and (ii) by protecting and stabilizing macromolecules and structures (i.e. proteins, membranes, chloroplasts, and liposomes) from damage induced by stress conditions (Crowe et al., 1992; Papageorgiou and Murata, 1995; Bohnert and Jensen, 1996). Similarly, high concentrations of many, but not all, compatible solutes (Rhodes and Hanson, 1993) have been proposed to confer protection against oxidative damage by scavenging free radicals as well as by maintaining osmotic equilibrium without altering macromolecule-solvent interactions.

Some osmolytes such as \( \beta \)-alanine (Rathinasabapathi, 2002), 3-dimethylsulphoniopropionate (Trosset et al., 1998) or glucosylglycerol (Bianchi et al., 1993) accumulate only in a limited number of families while others, especially proline and soluble sugars, are widespread among the plant kingdom. Glycinebetaine is absent in some important crop species such as rice or tomato, but accumulates to high amounts under salt or water stress conditions in plants belonging to the family of Chenopodiaceae (Hanson et al., 1985; McCue and Hanson, 1990). This is especially the case in the genus Atriplex; Atriplex hortensis has successfully been used as a source of gene coding for betaine aldehyde dehydrogenase that converts betain aldehyde into glycinebetaine for transgenic approaches in rice (Guo et al., 1997), tobacco (Shen et al., 2002), or tomato (Jia et al., 2002).

In some plant species, accumulation of glycinebetaine and proline may occur concomitantly (Colmer et al., 1995; Girija et al., 2002) but the physiological significance of this co-accumulation remains unclear since glycinebetaine may reduce the extent of proline accumulation (Gibon et al., 1997). In the Mediterranean xero-halophyte shrub Atriplex halimus, proline, quaternary ammonium compounds, and soluble sugars accumulate in both cell lines and whole plants exposed to salinity (Bajji et al., 1998). No data, however, are available concerning drought stress, although this species is not an obligate halophyte and is present in the absence of salt. It was recently shown that a population issued from an inland desertic area displayed a higher ability for OA in drought conditions than a population originating from a salt-affected coastal site (Martınez et al., 2003). These contrasting populations provide interesting material with which to (i) quantify the relative contribution of various osmolytes to OA and (ii) to determine the importance of OA in the adaptative response of Atriplex halimus to water stress.

Material and methods

Plant material and culture conditions

Fruits (seeds with enclosing bracts) of Atriplex halimus L. wild plants growing in the regions of Kairouan (dryland area from Tunisia with no salt contamination) and Tensift (NaCl-affected coastal site from Morocco) were used in this study. More than 800 seeds per population were surface sterilised for 30 s in 97% ethanol (v/v) followed by treatments in 0.8% (v/v) formaldehyde for 40 min and 5% (w/v) calcium hypochlorite for 20 min and then rinsed three times with sterile deionised water. Seeds were germinated on two layers of Whatman no. 41 filter paper moistened with 10 ml of sterile deionised water. Seeds were germinated on two layers of Whatman no. 41 filter paper moistened with 10 ml of sterile deionised water in the dark at 28°C. After 24 h, the seeds were transferred on a mixture of compost and sand (1/1) in a growth chamber for 7 days at 28/20°C (day/night) with a photoperiod of 16 h. Illumination was provided by Sylvania fluorescent tubes (F36 W/133-T8/CW) at a photon flux density of 170 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Uniform sized 7-day-old seedlings were transferred and acclimated in a greenhouse at 28/20°C (day/night) under a photoperiod of 16 h consisting of natural daylight supplemented with Phillips mercuric lamps (HPLN 400 W) to reach a minimum photon flux density of about 250 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Daytime humidity was about 70%. Twenty-six days after sowing, the young seedlings were transferred individually to polyethylene pots (15 x 10 cm²) each filled with 1.5 kg.
of sandy-textured soil with gravimetric water content at a field capacity of 8%. The soil surface was covered with a 4 cm gravel layer in order to avoid water losses by evaporation. No water restriction was applied to the plants for about 40 days after sowing. During this period, the plants were watered with Hoagland nutrient solution once a week. Subsequently, plants were randomly distributed within two groups, to which different water treatments (60 pots per treatment) were applied: (a) "Control" plants were watered to saturation every 7 days, and (b) "Water stress" was initiated by withholding water for 22 days. Soil water content, growth, water relations, glycinebetaine, and soluble sugar concentrations were evaluated 1, 8, 15 and 22 days after the treatment was initiated.

**Soil water content**

Soil gravimetric water content was defined as $\theta = Ww/DWs \times 100$ (where $Ww$ is the weight of the water contained in a soil sample, and $DWs$ is the dry weight of the sample). It was measured by the method of responding to changes in apparent dielectric constant using the ThetaProbe soil moisture sensor type ML1 (Delta-T Devices Ltd., dielectric constant using the ThetaProbe soil method of responding to changes in apparent dry weight of the sample). It was measured by the water contained in a soil sample, and $DWs$ is the dry weight determined after 48 h in an oven at 80°C. For $\psi$s, leaves were quickly collected, cut into small segments, then 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 tissular sap was analysed for $\psi$s estimation. Osmolarity (c) was assessed with a vapour pressure osmometer (Wescor 5500) and converted from mosmoles kg$^{-1}$ to MPa using the formula: $\psi s$ (MPa) = $-c$ (mosmoles kg$^{-1}$) $\times$ 2.58 $\times$ 10$^{-3}$ according to the Van’t Hoff equation. For the measurement of osmotic potential at full turgor ($\psi$s$^{100}$), leaves of stressed and control plants were rehydrated in deionised water for 24 h at 4°C in the dark. Total OA (OA$^{tot}$) was calculated as the difference of osmotic potential at full turgor between control ($\psi s_{c}^{100}$) and water stress ($\psi s_{s}^{100}$) treatments for each population (Blum, 1989; Zhang et al., 1999):

$$OA^{tot} = \psi s_{c}^{100} - \psi s_{s}^{100}.$$  

Solute concentration resulting from changes in non-osmotic volume due to insoluble polymer accumulation at full hydration OA$^{conc}$ was estimated using a modified formula (Girma and Krieg, 1992) from changes in TW/DW between control (c) and water stressed (s) plant leaves:

$$OA^{conc} = \frac{[(TW/DW)^{c} - (TW/DW)^{s}]}{[(TW/DW)^{s}]}OA.$$  

The contribution of net solute accumulation (OA$^{acc}$) was calculated as:

$$OA^{acc} = OA^{tot} - OA^{conc}.$$  

For determination of RWC, $\psi$s, OA$^{tot}$, OA$^{acc}$ and OA$^{conc}$, 10 plants per treatment were used.

**Organic solute quantification**

Old and young leaves of the main stem were analysed separately on 20 plants per treatment, for both levels containing the same number of leaves (when the main stem bore an uneven number of leaves, the median leaf was attributed to old leaves). However, for all analysed compounds except starch, there was no significant impact of the leaf age on endogenous concentrations and most data presented hereafter are pooled for both levels. All samples were frozen in liquid nitrogen and stored at −80°C until analysis.

Free proline was specifically quantified according to Bates et al. (1973). For glycinebetaine,
lyophilised material (50 mg) was extracted three times with 750 μl of 1 N HCl at 4°C. After centrifugation at 12,000g for 20 min at 4°C, the three supernatants were combined. The extract was filtered and dried by vacuum distillation. It was then dissolved in D2O to perform glycinebetaine determination through H1-NMR spectroscopy according to Jones et al. (1986), and using tert-butyl alcohol as an internal reference. NMR spectroscopy measurements were carried out with a 500.13 MHz Bruker AM 500 spectrometer. Presaturation of water was performed at room temperature with a recycling time of 7.7 s (5 s relaxation delay and 2.7 s acquisition time). The integrated intensities of the methyl resonances of the two compounds were identical within the experimental error (5%). Fourier transform conditions were: 3 kHz spectral width, 16K data points, 5 μs (45°) pulse width, 7.7 s recycle time, and 32 scans. A line broadening of 0.4 Hz was applied to the free induction decays before Fourier transformation. The solvent signal was suppressed by presaturating the H2O resonance.

Soluble sugars were extracted in 80% ethanol from 1 g of fresh leaf tissue. After centrifugation for 10 min at 8000g, the pellet and the supernatant were stored up to analysis. Total soluble sugar content was determined in leaves of ten plants per treatment by the classical anthrone method (Yemm and Willis, 1954) using a spectrophotometer (Beckman DU® 640, USA). Starch content was evaluated by a modified method of McCready et al. (1950). Starch remaining in the residue from ethanol extraction was hydrolysed to glucose using 16 ml 1 N HCl and incubated in a water bath at 95°C for 2 h. After filtration (Miracloth), pH was neutralised by adding 1 N NaOH and each sample volume was adjusted to 25 ml with deionised water, and starch was then determined colorimetrically using glucose as a standard.

Since the water stress had a significant effect on the RWC of the plants (see Fig. 2), proline, glycinebetaine, and sugar contents were adjusted to the RWC of unstressed plants (Z) according to $X \times Y/Z$, where X is the solute content and Y is the RWC of the stressed plants.

Statistical analysis

Data were analysed using a three-way analysis of variance (ANOVA) at a significance level of $P \leq 0.05(*)$ or $P \leq 0.01(**)$. The model is defined on the basis of fixed effects and hierarchal classification criterion. Main effects were considered to be population, treatment, and time, as well as their interaction. For starch, leaf age was considered as another source of variation. When the ANOVA was significant at $P \leq 0.05$, Duncan multiple range test was used for mean comparison. The data were analysed by a MSTACT statistical package. The statistical analysis showed that there were no significant differences between the results of two independent experiments. Data presented hereafter are taken from one experiment.

Results

Soil water content and plant growth

During the development of water stress, soil water content ($\theta$, %) decreased progressively and similarly in pots of the two populations ($F = 2502.1 **$). At the end of the experiments, $\theta$ of the stressed plants was about 3%, as compared to 24% in control plants.

In control conditions, the shoot elongated during the experimental period, reaching a maximum length of 29 cm in Kairouan and 34 cm in Tensift (Fig. 1). In water stress conditions, a significant reduction was observed in both populations ($F = 40.2 **$) (Fig. 1). It was detected earlier and was more marked in Tensift than in Kairouan. In contrast, leaf number at the end of the experimental period was similar in the two treatments for both populations, indicating that leaf production was not affected by the stress up until the time the experiment was discontinued (Fig. 1). Shoot DW increased similarly in unstressed plants of both populations (Fig. 1). This increase was slowed down by the water deficit after the 8th day of treatment, but the difference between control and stressed plants was significant only at the 22nd day ($F = 14.04 **$). Tensift was significantly less affected than Kairouan by the water stress.

Water relations

In controls, leaf RWC and $\Psi$s remained relatively constant during the experimental period (Fig. 2). In stressed plants, RWC and $\Psi$s declined markedly in both populations after the 8th day ($F = 36.20 **$ and $F = 30.44 **$, respectively) (Fig. 2); $\Psi$s reduction was larger in Kairouan than in Tensift ($F = 4.14{*}$) at the end of the experiment (Fig. 2). TW/DW in unstressed plants decreased significantly throughout the experimental period whatever the population. However, in stressed plants, the reduction of TW/DW was more marked in Kairouan than in Tensift after 22 days of water withholding (Fig. 2). Our results also show that during the period of water stress, OA tot increased in both
populations after the 8th day ($F = 22.55 \times 10^{-6}$) (Fig. 3), with a larger increase in Kairouan than in Tensift ($F = 3.91 \times 10^{-6}$) (Fig. 3). The contribution of net solute accumulation (OAacc) was slightly higher in Kairouan than in Tensift. The main difference however concerns contribution of changes in non-osmotic volume which was clearly higher in Kairouan than in Tensift at the end of the stress period.

**Osmolytes accumulation**

Proline concentration (Table 1) strongly differed between populations but it remained unchanged in response to water stress regardless of the population, with mean values of 1.3 and 4 $\mu$mol g$^{-1}$ FW in Kairouan and Tensift, respectively. The glycinebetaine concentration of unstressed plants increased progressively as a function of time in both populations (Fig. 4). In stressed plants, the increase was larger than in unstressed plants after the 8th day, being significant after 22 days of water withholding ($F = 34.19 \times 10^{-6}$). At this time, Tensift presented higher glycinebetaine concentrations than Kairouan.

Total soluble sugar concentrations of unstressed plants was slightly higher in Tensift than in Kairouan ($F = 17.56 \times 10^{-6}$) (Fig. 4) and rose slightly until the
end of the experimental period. In response to the stress, sugar content increased more rapidly after the 8th day in both populations. This increase was highly significant after 22 days of water withholding ($F = 3.7^{**}$). The ANOVA showed that the interaction between populations and water stress treatment was not significant. Although starch content of young and old leaves was higher in Tensift than in Kairouan under control conditions, the difference between the two populations was not significant. Starch content decreased in stressed plants regardless of population ($F = 8.4^*$), but the interaction between treatment and population was not significant.

**Discussion**

The way the stress was applied in this work allowed a relatively gradual development of water deficit. The dehydration of the plant tissues, caused by this treatment and revealed by the decrease in leaf RWC (Fig. 2), was delayed, however, until after the 8th day of treatment and was similar in both populations. As a result, no one measured parameter was affected by the treatment before that time.

Growth evaluated on a shoot DW basis was reduced in both populations. Tensift presented a tendency to reduce its shoot DW less than Kairouan.
and this result thus corroborates previous findings that demonstrated that water stress induces a higher decrease of CO₂ assimilation rates in Kairouan than in Tensift (Martínez et al., 2003). Shoot height also decreased significantly. The inhibition of growth, as indicated by shoot height and shoot DW (Fig. 1) was recorded when leaf RWC and θ were lower than 75% and 10%, respectively. The inhibitory effect of a water stress upon stem elongation and biomass accumulation is well documented in the literature (Mullet and Whitsitt, 1996; Nonami et al., 1997; Nonami, 1998). Unlike shoot height and shoot DW, leaf number was not affected in response to water stress, regardless of population (Fig. 1). Such a result could indicate that the activity of the meristem was preserved during the stress period and the question thus arises as to whether this is due to a higher tolerance of cell division to dehydration in comparison to cell elongation, as suggested by Kramer and Boyer (1995), or to a better protection against dehydration of the apical bud where higher RWC would be maintained in comparison to older organs and tissues. Another possibility would be that the lag time between primary events occurring in the meristematic tissues and the emergence of the leaves is too long to allow the detection of any effect upon leaf number in a short experimental period.

It has been frequently suggested that the aim of OA is to maintain growth capacity through turgor maintenance at lower external osmotic potentials. The present study demonstrates that there is no obvious correspondence between the OA and the biomass production in Atriplex halimus under our water stress conditions. Indeed, leaf Cs declined

Table 1. Effect of water stress on proline and starch concentrations (μmol g⁻¹ FW) in young and old leaves of two populations of Atriplex halimus (Kairouan and Tensift) under control and water stress treatments

<table>
<thead>
<tr>
<th>Populations</th>
<th>Leaf age</th>
<th>Treatment</th>
<th>Proline</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kairouan</td>
<td>Young</td>
<td>Control</td>
<td>12.2±0.7a</td>
<td>65.0±6.5a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water stress</td>
<td>14.1±1.1a</td>
<td>43.8±5.0bd</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>Control</td>
<td>13.7±1.2a</td>
<td>52.4±5.5bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water stress</td>
<td>11.9±0.5a</td>
<td>36.2±4.5c</td>
</tr>
<tr>
<td>Tensift</td>
<td>Young</td>
<td>Control</td>
<td>4.6±0.3a</td>
<td>74.0±5.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water stress</td>
<td>5.3±0.9a</td>
<td>56.9±7.5b</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>Control</td>
<td>3.7±0.4a</td>
<td>64.9±4.7a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water stress</td>
<td>3.9±0.5a</td>
<td>45.4±5.7bc</td>
</tr>
</tbody>
</table>

Measurements were done after 22 days of withholding water for stressed plants. The values are adjusted to the leaf RWC of controls of each provenance. Each value represents mean±SE (n = 10). Values sharing a common letter in each column are not significantly different at P≤0.05.
consistently in response to water stress in both populations, but the reduction was larger in Kairouan at the end of the experimental period (Fig. 3). Decreased $\Psi$s are generally considered to be an indicator of OA through the production and/or accumulation of so-called compatible osmolytes, although such decreases could also result from a dehydration of the tissue and/or a reduction of the osmotic volume. A dehydration process was revealed by the reduction of the leaf RWC we recorded, but it is insufficient to account for the $\Psi$s difference existing between both populations at the end of the experimental period (Fig. 3). We found indeed that, after eliminating the effect of tissue dehydration on $\Psi$s and solute accumulation of the sap of leaves submitted to the stress by adjusting leaf $\Psi$s at a defined water status (full turgor) (Blum et al., 1996), OA$^{\text{tot}}$ was still higher in Kairouan than in Tensift and was, on average 1.5 and 0.7 MPa for each population, respectively (Fig. 3).

Similarly, after eliminating the effect of solute concentration due to reduced osmotic volume, it still appeared that OA$^{\text{acc}}$ was slightly higher in Kairouan than in Tensift under water stress conditions (Fig. 3). Reduced osmotic volume could be the result of an increase in insoluble polymer accumulation, which is reflected in the turgid weight to dry weight ratio (TW/DW) (Girma and Krieg, 1992; Patakas and Noitsakis, 1999). The increase in OA due to change in TW/DW is not considered to be an active OA (OA$^{\text{acc}}$). In Atriplex halimus leaves, it was observed that the TW/DW decreased significantly more in Kairouan than in Tensift during the water stress period (Fig. 3). Since starch content was reduced in response to water stress (Table 1), the TW/DW should be due to an accumulation of other polymers such as hemicellulose and cellulose, as...
was reported for wheat (Wakabayashi et al., 1997). Such a modification in the composition of the cell wall may be involved in turgor maintenance through changes in wall elasticity. The situation in this respect, however, is not clear. In species that show OA and accumulate significantly high solute concentrations, a rigid cell wall may be necessary to maintain cell/tissues integrity on rehydration following a period of stress, and rigid cell walls may facilitate the maintenance of lower water potential at any given volume better than elastic walls (Clifford et al., 1998). In contrast, woody species with elastic cell walls were reported to have a high inherent drought tolerance in the absence of OA because in this situation, turgor potential is maintained over a wide range of RWC (Fan et al., 1994).

It has been shown that, for a given plant material and a given water stress intensity, the extent of OA varies depending on external parameters such as light (Delpérée et al., 2003) or N nutritional status (Ashraf et al., 2001). Thus, at this stage, the absence of a close relationship between OA and water stress resistance estimated in terms of growth in Atriplex halimus is valid under our experimental conditions only.

In the present work, the experimental evidence indicates that proline is not involved in OA of water stressed A. halimus plants since its content in leaves was relatively low and was not modified in response to the water deficit, although leaf RWC and $\Psi$s were significantly decreased. In salt stress conditions, Bajji et al. (1998) observed increased proline levels in leaves of A. halimus. This finding could suggest that alterations due to water deficit are less important than damages caused by salt or perhaps that Na$^+$ and Cl$^-$ ions may have additional toxic effects directly triggering proline overproduction independent of the osmotic component of salt stress. An impact of specific ions on proline-synthesizing enzymes was indeed reported in some experimental systems (Rout and Shaw, 1998; Girija et al., 2002). It is interesting to note that seeds of Tensift were collected on a salt-affected site while those of Kairouan originated from a non-saline dry area. Seedlings from those populations did not differ for their abilities to accumulate proline under drought conditions in the absence of NaCl.

In contrast, glycinebetaine concentration increased in leaves of A. halimus in response to the water stress treatment. The accumulation of this solute has been frequently reported under saline and water stress conditions and has usually been considered to have important physiological roles in the osmoregulation of the cytoplasmic cell compartment, in protein protection, and in membrane stabilization (Sakamoto and Murata, 2000 and references therein). From our results, we estimated that the quantitative contribution of glycinebetaine to the OA$^{exc}$ process in response to water stress at the whole leaf level was limited to less than 1%, regardless of the population. Under salinity conditions, total quaternary ammonium compound levels were found to increase in A. halimus leaves (Bajji et al., 1998), but once again, their estimated contribution to OA was very low (no more than 0.3%). However, because glycinebetaine, the major quaternary compound in chenopods, is predominantly localised in chloroplasts (Hanson et al., 1985; Rhodes and Hanson, 1993), the concentration inferred here greatly underestimates the levels in intact organelles. In photosynthetic systems, for instance, glycinebetaine efficiently protects various components of the photosynthetic machinery, including 1,5 bisphosphate carboxylase/oxigenase (Rubisco) and oxygen evolving photosystem II (PSII) complex, from salt-induced inactivation and disassociation into subunits (Papageorgiou and Murata, 1995; Allakhverdiev et al., 2003). Interestingly, the increase in glycinebetaine content was larger in Tensift, where photosynthesis activity was less affected by the water stress, than in Kairouan as was recently shown by Martínez et al. (2003).

The accumulation of sugars in plants in response to water stress is also quite well documented and is considered to play an important role in OA (Kameli and Lösel, 1995; Hare et al., 1998; Bajji et al., 2001). In the case of Atriplex halimus, the leaf-soluble sugar concentrations increased in both populations at a rate closely corresponding to the decrease in leaf RWC (Figs. 3 and 4). From a quantitative point of view, the contribution of soluble sugars to OA would be significant and represent, if all soluble sugars were hexoses, 44% and 68% to OA$^{exc}$ in Kairouan and Tensift, respectively, if expressed on a water content basis. However, the actual contribution of sugars to $\Psi$s should be lower, since disaccharides should also be present. Decrease of starch levels when water availability is limited has been observed in leaves of many different plant species (Geigenberger et al., 1997; Geiger et al., 2000). The decrease in starch content could result from a decrease in synthesis, since $\mathrm{CO}_2$ assimilation was reduced in response to water stress as shown by Martínez et al. (2003). However, this reduction could also be caused by a stimulation of starch degradation, regulated by hydrolytic and/or phosphorolytic pathways (Geiger et al., 2000). For example, Todaka et al. (2000) observed that beta-amylase activity in cucumber cotyledons increased in response to PEG water stress.
In conclusion, this study demonstrates that OA is not an absolute pre-request for water stress resistance in *Atriplex halimus*. It also provides some evidence supporting the hypothesis that accumulation of sugars plays a preponderant role in the contribution to OA in this species while glycinebetaine plays a minor role in OA, but is probably associated with the protection of enzymes and cellular structures.

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