Ammonium Nutrition Improves Salt Tolerance of Spartina alterniflora

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ABSTRACT

The effects of inorganic nitrogen (N) form on biomass allocation, photosynthesis, osmotic adjustment, and cell wall elasticity of Spartina alterniflora were studied in a greenhouse under natural light and with sandy soil in the presence or absence of 500 mM NaCl. Under non saline conditions ammonium nutrition enhanced dry matter production and leaf area by ca. 50% in comparison to nitrate-fed plants. Under saline conditions the enhancement was more pronounced. NH4+ nutrition improved photosynthesis, transpiration, and stomatal conductance, suggesting that S. alterniflora prefers ammonium as a nitrogen source. The addition of 500 mol.m-3 NaCl significantly affected plant growth in both nitrate and mixed media, but had no impact when ammonium was supplied alone, suggesting a considerable advantage of NH4+ nutrition under saline conditions. As a whole, ammonium treatment seems to improve salt tolerance of S. alterniflora by decreasing the energetic cost, needed for osmotic adjustment and cell wall elasticity, to growth.

Keywords: cell wall elasticity, gas exchange, halophyte, osmotic adjustment, salinity

INTRODUCTION

Salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semiarid region of the world, reducing the value and productivity of the affected land (Munns and Tester 2008; Mhamedin et al. 2010). Soil infertility is often due to the presence of large quantities of salt, and the introduction of plants capable of surviving under these conditions is highly interesting (Hessini et al. 2009a; Hamdia et al. 2010).

Because of their immobility, plants have evolved complex internal systems to adapt to the changing and often stressful environments. Of these systems, osmotic adjustment and cell wall elasticity (CWE) are two important physiological mechanisms of adaptation to internal and external constraints. Plants need to maintain internal water potential below that of soil to maintain turgor and sufficient water supply for growth. This requires the maintenance of low osmotic potentials, either by uptake of solutes from the soil or by synthesis of metabolically compatible solutes (Tester and Davenport 2003; Hessini et al. 2009b). Compatible solutes, such as soluble sugars and organic-N compounds, are known to accumulate under conditions of environmental stresses and to play a role in the process of osmotic adjustment in many crops (Hessini et al. 2009b).

The impact of N nutrition on salt tolerance has been analysed at different levels of N metabolism such as N uptake, assimilation and N (total N, proteins and amino acids) accumulation (Ehlting et al. 2007). It is thought that sufficient N supply helps to compensate and correct nutritional imbalances in salt-stressed plants (Gomez et al. 1996), as salt stress decreases the uptake of nutrients such as NO3- (Ehlting et al. 2007; Hessini et al. 2009c).

Limited information is available on plant performance as a function of nitrogen source (nitrate or ammonium) upon exposure to salt stress. In a salt marsh of Barataria Bay (Louisiana), both yield and nutrient uptake of S. alterniflora were enhanced by increased ammonium supply (Patrick and Delaune 1976). Similar results in S. alterniflora were found with ammonium that showed the critical nitrogen concentration is a function of salinity and indicated that the internal nitrogen supply required for growth increases with salinity (Bradley and Morris 1992). Analysing growth rate and gas exchange, Lewis et al. (1989) suggested that ammonium-fed plants are more susceptible to salt. In different herbaceous species (Pisum sativum, Helianthus annuus, Glycine max), nitrate-supplied plants were less sensitive to salt stress compared with ammonium-supplied individuals with regard to mineral composition, growth and physiological parameters (Bourgeais-Chaillou et al. 1992; Ashraf and Sultan 2000; Frechilla et al. 2001).

Spartina alterniflora can be grown on saline soil in order to prevent erosion and to possibly forage production (Chung et al. 2004; Hessini et al. 2009b). We had previously demonstrated that ammonium treatment improved salt tolerance of this species at high salinity (500 mM NaCl) by increasing secretion activity of salt glands, thereby decreasing salt contents of leaves and avoiding toxic buildup of Na+ in the leaf apoplasm (Hessini et al. 2009c). The purpose of the present work was to examine whether other physiological mechanisms may explain such an improvement.

MATERIALS AND METHODS

Plant material, growth conditions and stress imposition

Seeds of Spartina alterniflora Loisel from its native range (USA) were sown in five outdoor containers filled with a mixture of sandy soil and organic matter at the Experimental Station of Borj-Cedria Biotechnology Center (Tunisia) during the spring-summer
Table 1 Variation of pH in different extract solutions during the experiment period.

<table>
<thead>
<tr>
<th>NO\textsubscript{3}\textsuperscript{-}NH\textsubscript{4}\textsuperscript{+}</th>
<th>0 (day)</th>
<th>10 (days)</th>
<th>20 (days)</th>
<th>30 (days)</th>
<th>40 (days)</th>
<th>50 (days)</th>
<th>60 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100/0</td>
<td>6.25</td>
<td>6.55</td>
<td>6.65</td>
<td>6.95</td>
<td>6.80</td>
<td>7.05</td>
<td>7.2</td>
</tr>
<tr>
<td>0/100</td>
<td>6.26</td>
<td>5.89</td>
<td>6.43</td>
<td>6.75</td>
<td>6.50</td>
<td>6.55</td>
<td>6.8</td>
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<tr>
<td>500 mM NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100/0</td>
<td>6.07</td>
<td>6.62</td>
<td>7.09</td>
<td>6.95</td>
<td>7.21</td>
<td>6.90</td>
<td>7.05</td>
</tr>
<tr>
<td>0/100</td>
<td>6.05</td>
<td>5.95</td>
<td>6.71</td>
<td>6.50</td>
<td>6.50</td>
<td>6.90</td>
<td>6.60</td>
</tr>
</tbody>
</table>

Leaf water potential and water content

Leaf water potential (Ψw) and water content (WC) were measured at 10:00 am on 7 to 10 fully expanded leaves per treatment at 60 DAT. Leaf water potential (Ψw) was measured using a pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA, USA) (Scholander et al. 1965). Leaf WC was calculated as follows:

\[ WC = \frac{(FW - DW)}{FW} \times 100 \]  

where FW corresponds to the leaf fresh weight, DW to the leaf dry weight (72 h at 80°C).

Osmotic potential and cell wall elasticity

The pressure chamber was also used to obtain pressure-volume curves from leaves of each treatment. Measurements were taken during 2 days between 56 and 58 DAT. Fully expanded leaves were removed by cutting under distilled water, and re-hydrated for 18 h at 4°C in a dark humid chamber. Pressure-volume curves were generated using the repeated pressurisation technique, with leaves weighed on a precision balance between measurements of pressure (Tyrre and Hamnel 1972). After establishing the balancing pressure, the chamber pressure was successively raised by 0.2 MPa increments. The elevated pressure was sustained for 10 min. After each sap collection, the chamber pressure was slowly reduced to the previous balance pressure. The new balance pressure was then determined. The pressurisation rate was about 0.025 MPa s\textsuperscript{-1}. Osmotic potential at full turgor (Ψ\textsubscript{T}) and osmotic potential at zero turgor (Ψ\textsubscript{0}) were determined according to Sinclair and Venables (1983) using the linear regression of Ψ\textsubscript{k} versus (1-RWC). The degree of osmotic adjustment (OA) was defined as the difference in Ψ\textsubscript{k} between the control (Ψ\textsubscript{k0}) and the stressed plants (Ψ\textsubscript{k}).

\[ OA = \Psi_{k0} - \Psi_{k} \]  

The cell wall elasticity (CWE) of leaf tissue was estimated through the determination of the elasticity modulus (ε) value. This parameter was determined according to Hessini et al (2009b) as:

\[ ε = \frac{(Ψ_{k0} - Ψ_{k})}{(1 - AWC)} \]  

where AWC is the apoplastic water content and RWC\textsubscript{0} is the relative water content at zero turgor.

Statistical analysis

All data presented are mean values. The measurements were achieved on five replicates for shoot ionic content and ten replicates for biomass determination and water content. Statistical analyse (one-way ANOVA) test was carried out using the SPSS 16.0 software. In case of significant differences, means were compared by the Duncan’s multiple range tests at P < 0.05.

RESULTS AND DISCUSSION

Plant growth and gas exchange

Plant growth was assessed by the whole plant dry weight (DW), the leaf area and leaf number (Table 2). Under non-saline conditions, the highest DW was registered in the medium containing both nitrate and ammonium, whereas the lowest DW was found for plants grown with nitrate as the only N source. Yet, growth was better under ammonium than nitrate supply, indicating that S. alterniflora is one of the scarce species preferring ammonium as nitrogen source. The beneficial effect of NH\textsubscript{4}\textsuperscript{+} over NO\textsubscript{3}\textsuperscript{-N} was also reported for some wetland and marine species (Thursby and Harlin 1984; Munzarova et al. 2006; Hessini et al. 2009c) as well as for plants colonising terrestrial habitats where NH\textsubscript{4}\textsuperscript{+}-N form is prevailing (Munzarova et al. 2006; Dennis and Hans 2010).

Adding 500 mM NaCl to the different media led to a re-

season of 2005. The experimental station is located near the Mediterranean seashore, 35 km north-east of Tunis (10°10'E, 36°48'N; 10 m altitude). For the spring-summer period, the average daily temperature and cumulated rainfall were 20°C and 218 mm, respectively. About 200 plants were grown in each outdoor container (1 m\textsuperscript{3}) and were irrigated weekly with tap water. Uniform cuttings (25 cm height) were taken from these mother plants and were washed before individually transplanting into 4 L pots (one plant plug per pot) filled with sandy soil and weekly irrigated with modified Hewitt’s solution (Hewitt 1966) [0.55 mM phosphorus (P), 1.45 mM potassium (K), 0.37 mM magnesium (Mg), 0.87 mM calcium (Ca), 10.0 μM boron (B), 1.0 μM manganese (Mn), 0.5 μM zinc (Zn), 0.5 μM copper (Cu), and 0.35 μM molybdenum (Mo)] under non-saline condition for the first month after transplanting. Three-day-old cuttings of S. alterniflora were then cultivated in a greenhouse under natural light, with an average temperature of 25/18°C day/night and a relative humidity of 65/90%. Four-fold diluted nutrient solution (250 mL per pot) providing macro- and micronutrients was incorporated in the irrigation system at three day-intervals, providing equal amounts of nutrients to each plant. The volume of irrigation water represented 100% of field capacity (FC), which was measured according to the technique of Bouyoucos (1983). Nitrogen (7.5 mM) was applied either as NO\textsubscript{3}\textsuperscript{-} (100/0), NH\textsubscript{4}\textsuperscript{+} (0/100) or a mixture of NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} (50/50) with or without 500 mM NaCl. Salt was added weekly in steps of 100 mM in order to avoid an osmotic shock. The medium containing NH\textsubscript{4}\textsuperscript{+} as the only N source was buffered with 0.33g CaCO\textsubscript{3} per Kg DW of soil (Cantera et al. 1999). The data about the culture solution pH are given in Table 1. Evaporation from the pots surface was reduced by enclosing all pots in plastic bags sealed at the base of the rhizome of each cutting.

Growth

The fresh (FW) and dry matter (DW) of shoots and roots of each plant were determined after counting the number of leaves and determining leaf surface area with portable area meter (LI-3000A). These measurements were performed between 9:00 and 10:00 AM. The dry matter weights were determined after drying shoots and roots for 72 h in a thermo-ventilated oven at 80°C. Measurements were performed on ten replicates per treatment 60 days after beginning of treatment (DAT).

Gas exchange measurements

Gas exchange parameters were determined using a portable gas exchange system (Li-Cor 6200, Li-Cor Nebraska USA). Measurements were first performed under increasing light intensities (PAR from 0 to 2500 μmol m\textsuperscript{-2} s\textsuperscript{-1}) and showed that net CO\textsubscript{2} assimilation rate (A) was maximal at 2000 μmol m\textsuperscript{-2} s\textsuperscript{-1} (data not shown). Subsequently, measurement conditions were the following: 2000 μmol m\textsuperscript{-2} s\textsuperscript{-1} PAR (saturating light intensity), 350 μmol mol\textsuperscript{-1} ambient CO\textsubscript{2} concentration; 29 ± 2°C leaf temperature. Measurements were carried out between 10:00 and 12:00, on leaves (10 replicates per each level of water logging) acclimated for 15 min to the leaf chamber conditions. The following parameters were registered at saturating light: net CO\textsubscript{2} assimilation rates (A), stomatal conductance (gs), transpiration rate (E), and transpiration efficiency (ETE) as the ratio E/A. The gas exchange measurements were made at 58 DAT.
Table 2 Effect of nitrogen ionic form and salinity on dry weight (DW, g plant$^{-1}$), leaf area (cm$^2$, plant$^{-1}$), and leaf number in Spartina alterniflora.

<table>
<thead>
<tr>
<th>NO$_3$/NH$_4$</th>
<th>DW (g plant$^{-1}$)</th>
<th>Leaf area (cm$^2$, plant$^{-1}$)</th>
<th>Leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100/0</td>
<td>2.82 ± 0.85 ab</td>
<td>152.64 ± 89.74 ab</td>
<td>12.57 ± 3.17 b</td>
</tr>
<tr>
<td>50/50</td>
<td>6.02 ± 1.07 c</td>
<td>339.81 ± 120.52 d</td>
<td>16.67 ± 0.72 c</td>
</tr>
<tr>
<td>0/100</td>
<td>4.57 ± 1.74 bc</td>
<td>221.48 ± 85.94 bc</td>
<td>13.43 ± 4.25 c</td>
</tr>
<tr>
<td>500 mM NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100/0</td>
<td>2.35 ± 0.82 a</td>
<td>78.82 ± 17.34 a</td>
<td>8.25 ± 1.16 a</td>
</tr>
<tr>
<td>50/50</td>
<td>2.46 ± 0.87 a</td>
<td>147.78 ± 21.08 ab</td>
<td>7.1 ± 0.64 a</td>
</tr>
<tr>
<td>0/100</td>
<td>4.71 ± 0.94 bc</td>
<td>262.28 ± 41.59 e</td>
<td>16.75 ± 2.96 c</td>
</tr>
</tbody>
</table>

Lower case letters indicates differences (P<0.05, Duncan test) between treatments.

Table 3 Effect of nitrogen ionic form and salinity on photosynthesis (A, μmol CO$_2$ m$^{-2}$ s$^{-1}$), transpiration rate (E, mmol H$_2$O m$^{-2}$ s$^{-1}$) and stomatal conductance (gs, mmol H$_2$O m$^{-2}$ s$^{-1}$) in Spartina alterniflora.

<table>
<thead>
<tr>
<th>NO$_3$/NH$_4$</th>
<th>A</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100/0</td>
<td>12.48 ± 0.66 b</td>
<td>2.05 ± 0.26 b</td>
</tr>
<tr>
<td>50/50</td>
<td>18.12 ± 0.79 c</td>
<td>2.21 ± 0.36 cb</td>
</tr>
<tr>
<td>0/100</td>
<td>17.76 ± 0.94 c</td>
<td>2.55 ± 0.24 b</td>
</tr>
<tr>
<td>500 mM NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100/0</td>
<td>8.02 ± 1.19 a</td>
<td>1.02 ± 0.21 a</td>
</tr>
<tr>
<td>50/50</td>
<td>7.31 ± 0.61 a</td>
<td>1.26 ± 0.38 a</td>
</tr>
<tr>
<td>0/100</td>
<td>16.92 ± 0.45 c</td>
<td>2.21 ± 0.06 cb</td>
</tr>
</tbody>
</table>

Lower case letters indicates differences (P<0.05, Duncan test) between treatments.

Table 4 Effect of nitrogen ionic form and salinity on dry weight (DW, g plant$^{-1}$), leaf area (cm$^2$, plant$^{-1}$), and leaf number in Spartina alterniflora.

<table>
<thead>
<tr>
<th>NO$_3$/NH$_4$</th>
<th>DW (g plant$^{-1}$)</th>
<th>Leaf area (cm$^2$, plant$^{-1}$)</th>
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</thead>
<tbody>
<tr>
<td>0 mM NaCl</td>
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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>100/0</td>
<td>2.35 ± 0.82 a</td>
<td>78.82 ± 17.34 a</td>
<td>8.25 ± 1.16 a</td>
</tr>
<tr>
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<td>7.1 ± 0.64 a</td>
</tr>
<tr>
<td>0/100</td>
<td>4.71 ± 0.94 bc</td>
<td>262.28 ± 41.59 e</td>
<td>16.75 ± 2.96 c</td>
</tr>
</tbody>
</table>

Lower case letters indicates differences (P<0.05, Duncan test) between treatments.

Osmotic potential and cell wall elasticity

The effect of nitrogen form on the leaf water relations was also investigated. Irrespective of nitrogen form, the addition of 500 mM NaCl caused a reduction of water content, relative water content at turgor loss point and osmotic potential both at full turgor (Ψ$_t$) and at turgor-loss point (Ψ$_p$). However, this decrease was less pronounced in ammonium-fed plants than for the other treatments. The decrease of both osmotic potential at full turgor and at turgor-loss point (calculated from the pressure–RWC relationships) in salt stressed leaves compared with the control indicated an osmotic adjustment (Table 4). The role of the latter is to maintain growth capacity through turgor maintenance at lower external osmotic potentials (Gomes et al. 2010; Chen and Jiang 2010). However, the degree of osmotic adjustment was significantly affected by N-source; in fact, there is a close but negative correlation between the degree of osmotic adjustment and the NO$_3$/NH$_4$ ratio (Fig. 1), whereas there is a positive correlation for the growth parameter. This result indicated that there is an obvious correspondence between the degree of OA and the biomass production in S. alterniflora under our nitrogen treatments (Fig. 1). It seemed that the decrease of the osmotic adjustment by NH$_4^+$ in the salty medium led to positive effects on plant growth.

In addition to osmotic adjustment, the cell wall elasticity (CWE) is also considered one of the most important physiological mechanisms of adaptation to osmotic stress (Martínez et al. 2007; Hessini et al. 2009b; Singh et al. 2010). In our study, except in buffered ammonium medium, the addition of 500 mM NaCl induced a significant decrease in leaf cell elasticity (i.e., the bulk modulus of elasticity, ε$_{max}$, increased), which caused the turgor loss point to move to a higher RWC (Table 4). This result is in agreement with previous findings for olive tree (Karamanos 1984) and common bean plants (Stoyanov 2005; Sassi et al. 2010). An increase in ε$_{max}$ (stiffness) is expected when the cell walls become more rigid or thicker. The greater stiffness of the cell wall, given an equal decrease in cell volume and will be responsible for the drop in water potential. Except in nitrate fed-plants, for which the apoplastic water fraction was decreased, salinity significantly improved this parameter in both ammonium and mixed-media (Table 4). This change was reduced by an average of 50%. This suggests that in spite of the large decrease of stomatal aperture, salt-treated plants in ammonium media were able to maintain a high photosynthetic and transpiration rate. This result is in agreement with previous findings on barley (Kant et al. 2007) and tomato (Flores et al. 2001; Horchani et al. 2010). On the other hand, the growth increase observed in the present study with the ammonium regime could be correlated with an increase in both photosynthesis per unit area and total leaf area available for photosynthesis. The beneficial effect of ammonium on total leaf area confirm our previous results (data unpublished) that showed that ammonium nutrition affected only slightly individual leaf area of Beta macrocarpa, but had no significant effect on the initiation rates of leaves.
Fig. 1 Osmotic adjustment capacity and dry weight accumulation of salt-treated plants depending on nitrogen regime. Values are the means of five observations and bars are standard errors. Different letters on top of the symbols indicate statistically significant differences at P<0.05.

can contribute to passive concentration of solutes and thus to turgor maintenance in *S. alterniflora* plants grown on ammonium and mixed-media (Patakas and Noitsakis 1999). We concluded from our study of the cellular water relations that ammonium nutrition diverts the energetic cost, needed for osmotic adjustment and cell wall elasticity, to growth.

ACKNOWLEDGEMENTS

Authors would like to acknowledge the support of the COST action FA0901: Putting Halophytes to Work - From Genes to Ecosystems.

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