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Effects of salinity on growth, membrane permeability and root hydraulic conductivity in three saltbush species

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ABSTRACT

The species of the genus *Atriplex* have been introduced in West Asia and North Africa to determine their adaptability for use as fodder species. These halophytes are well adapted to extreme environmental conditions and may possess interesting properties for soil rehabilitation. The effect of NaCl stress on growth, water relation and mineral nutrition were investigated in three xero-halophyte species of *Atriplex* used for rehabilitation of arid steppe in Algeria. *Atriplex halimus*, *Atriplex canescens* and *Atriplex nummularia*, were cultivated in hydroponic conditions and treated with increasing doses of NaCl (0–300 mM). All species showed positive plant growth for low and moderate levels of salinity. *A. halimus* had higher dry weight production than *A. nummularia* and *A. canescens* in high salinity concentration. Increasing concentration of salinity induced decrease in chlorophyll content (Chl *a* and *b*) and root hydraulic conductivity (L_0) in all species, especially in *A. canescens*. All three species showed marked increase in electrolyte leakage across the salinity gradient. In addition all species were able to accumulate a large quantity of sodium (Na), chloride (Cl) and proline and to maintain higher relative water content, which was probably associated with a greater capacity for osmotic adjustment, whereas potassium (K) and calcium (Ca) decreased with increase salinity. The data suggest that salt tolerance strategies in all *Atriplex* species could involve a delicate balance among ion accumulation, osmotic adjustment, production of osmotica and maintenance of relative water content and growth.

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1. Introduction

Salinity affects more than 40% of soils in the Mediterranean basin. It is increasing steadily in many parts of the world due to poor irrigation and drainage practices, which cause a great reduction for crop productivity (Drevon et al., 2001). As an alternative method to restore saline land, the utilization of halophytes attracted more attention due to their salt tolerance characteristics and potential economic values (Khan and Gul, 2006; Nedjimi and Daoud, 2009a). The principal mechanism of halophytic adaptation may be the high Na and Cl absorption. The halophytes which are able to grow at high salinity can generate a high turgor in their cells by the high internal Na and Cl concentrations. They also have some adaptive features like salt secretion (salt glands & bladders) or increase succulence to deal with high concentration of ions in the cell (Flowers and Colmer, 2008).

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Atriplex halimus L. (Mediterranean saltbush) is a perennial shrub which grows throughout the Mediterranean basin and is used widely to provide forage, due to its drought and salt tolerance and its high protein content (Le Houérou, 1992). *Atriplex canescens* (Pursh.) Nutt. (Fourwing saltbush) is the most widespread saltbush in North America, found in saline and xeric soils from Mexico to Canada (Glenn and Brown, 1998). *Atriplex nummularia* L., commonly named old man saltbush, is a halophytic species that naturally occurs in the Australian desert regions (Silveira et al., 2009). *A. halimus* is native to the arid steppe of Algeria, whereas *A. canescens* and *A. nummularia* were introduced in 1987 from the USA and Australia respectively, as a source of fodder in pastoral plantations.

Numerous studies have investigated the effect of salt stress on *Atriplex* species, but few have compared the response to salt stress in these species to understand the mechanisms of salt tolerance. In this study, we have examined the effects of salinity on plant growth, water relation and ions accumulation in three *Atriplex* species over a range of salt concentrations, in order to better understand their differences on salt stress tolerance.

2. Materials and methods

2.1. Plant material and growth conditions

The seeds of *A. halimus*, *A. canescens* and *A. nummularia* were collected from the area of *El- Mesrane* in the province of *Djelfa* (Algeria) (chott *Zahrez* zone; 3°03′E longitude, 34°36′N latitude, and 830 m elevation). Seeds were sterilized for 30 s in 97% ethanol, followed by a treatment with 0.8% formaldehyde for 40 min and 5% calcium hypochlorite for 20 min. They were finally rinsed three times with sterile deionized water (Nedjimi and Daoud, 2009b). Seeds were pre-hydrated with aerated, deionized water for 12 h and germinated in vermiculite, at 28 °C in an incubator, for 2 d. They were then transferred to a controlled-environment chamber with a 16 h light – 8 h dark cycle and air temperatures of 25 and 20 °C, respectively. The relative humidity (RH) was 60% (day) and 80% (night) and photosynthetically-active radiation (PAR) was 400 μmol m^{-2} s^{-1}, provided by a combination of fluorescent tubes (Philips TLD 36 W/83, Germany and Sylvania F36 W/GRO, USA).

After 7 d, the seedlings were placed in 15-L containers (six plant per container) with continuously aerated Hoagland (Hoagland and Arnon, 1950) nutrient modified solution: 2.5 mM $Ca(NO_3)_2$, 2.5 mM KCl, 1.0 mM $MgSO_4$, 0.25 mM $Ca(H_2PO_4)_2$, 12.5 μM H_3BO_3, 1.0 μM $MnSO_4$, 1.0 μM $ZnSO_4$, 0.25 μM $CuSO_4$, 0.2 μM $(NH_4)_6Mo_7O_{24}$ and 10 μM Fe-EDDHA (Fe-ethylenediamine-di-o-hydroxyphenylacetic acid). Each treatment was replicated five times and each replicate included six plants (i.e. 30 plants per treatment). The pH was kept within the range of 6.5–7.0 by adding H_2SO_4 or KOH. The solution was replaced completely every week. After a further 13 d (when plants were 20 days-old), plants were treated with 0, 100, 200 and 300 mM NaCl. Dry weight, water content, root hydraulic conductance (L_0), chlorophyll, proline, ions and membrane permeability were measured after 30 d of the treatments (i.e. 50 d after germination).

2.2. Dry weight determination and chemical analysis

Plants were divided into shoots and roots, and dried in an oven at 65 °C for 72 h to determine dry weight (DW) and mineral contents. Chemical analyses were carried out on DW basis. Sodium (Na), potassium (K) and calcium (Ca) concentrations were determined from dry powdered plant tissue after extraction in HCl using an atomic absorption spectrophotometer (905AA, GBC, Australia). Chloride (Cl) was measured with Chromeleon/Peaknet 6.40 chromatography software, by comparing peak areas with those of known standards.

2.3. Relative water content (RWC)

Leaf relative water content (RWC) was estimated by recording the turgid weight which represents fully hydrated leaf weight by keeping the leaves in water for 4 h, followed by their drying in hot air oven till constant weight achieved (Ben Amor et al., 2005). The following equation was used for determining RWC:

$$\mathrm{RWC}(\%) = [\mathrm{FW} - \mathrm{DW}/\mathrm{TW} - \mathrm{DW}] \times 100.$$

where TW stands for turgid weight, FW fresh weight and DW dry weight.

2.4. Root hydraulic conductance (L_0)

Hydraulic conductance (L_0) of roots was measured by pressurising the roots using the Scholander chamber (Jackson et al., 1996). For this, the aerial parts of the plant were removed, leaving the base of the stem, which was sealed with silicone grease, into a tapered glass tube. The plant was placed into a pressure chamber, with the same nutrient solution that it was grown in, and a gradual increase of pressure (from 0.2 to 0.4 MPa) was applied to detached roots. The range of the generated sap flows included a flow equivalent to the whole-plant transpiration flow. In the pressure chamber, it is assumed that there is a balance between the negative pressure in the xylem and the pressure that forces water from the cells into the vessels. Sap was collected in Eppendorf tubes and weighed on a precision balance. Sap flow (J_V) was expressed in mg $(g_{root\ FW})^{-1}$ h^{-1} and plotted against pressure (MPa), the slope being the L_0 value (mg $[g_{root\ FW}]^{-1}$ h^{-1} MPa^{-1}).

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