



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

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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 (Ψ_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, Ψ_s , and TW/DW, but an increase in glycinebetaine and sugar leaf contents. The decrease of Ψ_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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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; Ψ_s , osmotic potential; RWC, relative water content; TW, turgid weight

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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 β -alanine (Rathinasabapathi, 2002), 3-dimethylsulphoniopropionate (Trosat 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 (Martinez 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 adaptive 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 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 Philips 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 ($15 \times 10 \text{ cm}^2$) each filled with 1.5 kg