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Differential responses of saltbush *Atriplex halimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene

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ABSTRACT

Drought and salinity induce water deficit, but may also have distinct effects on plant metabolism. To compare their impact on leaf senescence in relation to ABA and ethylene synthesis, young plants of *Atriplex halimus* L. were exposed to iso-osmotic concentrations of NaCl (160 mM) or PEG (15%) in nutrient solution. Plant growth and development were more affected by PEG than by NaCl. Stressed plants remained able to reduce their osmotic potential, but the nature of accumulated organic osmocompatible solutes varied according to the stressing agent. Glycinebetaine accumulated to a greater extent in salt-treated plants than in water-stressed plants. Sodium chloride induced the accumulation of non-reducing sucrose, while PEG-treated plants mainly accumulated reducing glucose and fructose. Abscisic acid (ABA) accumulated in response to salt, while ethylene was synthesized mainly by PEG-treated plants and was involved in the induction of early senescence processes characterized by synthesis of reactive oxygen species, peroxidation of membrane lipids and a decrease in chlorophyll content. ABA sensitivity of stressed tissues was markedly different in response to salt and in response to non-ionic osmotic stress, and exogenous ABA (50 μM) had contrasting effects on most physiological parameters depending on the stressing agent. Exogenous ABA induced a decrease in root and shoot growth and sucrose content, and an increase in reactive oxygen species content in salt-stressed plants. In contrast, exogenous ABA increased growth in PEG-treated plants in relation to an improvement of water use efficiency resulting from a more efficient stomatal control. Exogenous ABA increased ethylene synthesis in salt-treated plants, but had only marginal impact on PEG-treated ones. The xero-halophyte *A. halimus* thus responds in a contrasting way to salt and water stress, through accumulation of distinct osmocompatible solutes and hormonal compounds such as ethylene and ABA could play distinct roles in stress-induced senescence processes.

Introduction

Several environmental factors adversely affect plant growth and development. Salinity is one of the most deleterious abiotic stresses, and induces a wide range of perturbations at both the cell and whole plant levels (Munns, 2005). Drought is also considered an increasingly expensive problem for plant production (Araus et al., 2007). Plant responses to salt and water stress have much in common, since high salt concentrations decrease the osmotic potential of soil solution, creating water stress in plants. In addition to this "osmotic" constraint, salt stress also imposes ionic stress on plants, mainly in relation to Na^+ and Cl^- accumulation (Lefèvre et al., 2001; Munns, 2002).

Turgor regulation during changes in plant water status may preserve the metabolic processes of the plant and contribute to growth maintenance. Osmotic adjustment is defined as lowering of the osmotic potential (Ψs) due to net solute accumulation. Compatible solutes involved in such processes may act as (i) cytoplasmic osmolytes facilitating water uptake (Flowers, 2004), (ii) protectors and stabilizers of macromolecules and cellular structures for damage induced by stress conditions (Bohnert and Jensen, 1996; Hoekstra et al., 2001) and (iii) scavengers of free radicals against oxidative damage (Ashraf and Foolad, 2007). Some osmolytes, such as proline and soluble sugars, are widespread among the plant kingdom. In contrast, glycinebetaine is absent in some important crop species that are unable to synthesize it, but this quaternary ammonium compound accumulates in large amounts under salt or water stress conditions in plants belonging to the Chenopodiaceae family (Chen and Murata, 2008).

In addition to osmotic adjustment and protection of cellular structures, stomatal regulation controlled by abscisic acid (ABA) often appears an efficient strategy to reduce water deficit caused either by salinity or by drought (Ren et al., 2007). ABA moves freely

Abbreviations: ABA, abscisic acid; Chl, chlorophyll; DW, dry weight; FW, fresh weight; g_s, stomatal conductance; MDA, malondialdehyde; pAR, photosynthetically active radiation; Ψs, osmotic potential; Ψw, water potential; WC, water content.

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in the xylem and phloem and has also been shown to trigger ABA-responsive genes (Bray, 2002). In addition to its positive impact on stressed plant responses, ABA is also considered a senescing hormone and can thus assume dual functions in plant behavior (Ghanem et al., 2008). Salt-induced senescence involves a decrease in photosynthetic pigments and in cell membrane stability (Lutts et al., 1996). In several species, the involvement of ethylene in enhancing leaf senescence has also been demonstrated, especially under drought (Young et al., 2004) and salt stress (Wi and Park, 2002). It has been hypothesized that leaf senescence is the ultimate consequence of a global hormonal deregulation characterized by stress-induced modifications in senescing (ABA and ethylene) and anti-senescing (mainly cytokinin) hormones (Albacete et al., 2008; Ghanem et al., 2008). Nevertheless, stress-induced modifications in ABA and ethylene in xero-halophyte plant species remain poorly documented.

Atriplex halimus L. (Chenopodiaceae) is a monoecious C4 perennial shrub native to the Mediterranean basin with excellent tolerance to drought and salinity (Bajji et al., 1998; Martínez et al., 2003). 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). *A. halimus* has evolved various protective mechanisms allowing this species to survive and grow in different harsh environments and to respond to abiotic stress through a number of physiological mechanisms. It also displays a high level of variability both between and within populations (Ortíz-Dorda et al., 2005). It was recently demonstrated that there is a relationship between the habitat of *A. halimus* and the strategy adopted for water resistance; populations issued from a coastal salt area mainly accumulated glycinebetaine as osmoprotectors, while populations from a dry inland area preferentially accumulated proline (Ben Hassine et al., 2008). In both cases, however, total soluble sugars also accumulated and, from a quantitative point of view, similarly contributed to osmotic adjustment in both populations (Ben Hassine et al., 2008). However, the nature of accumulated sugars, as well as the involvement of ABA and ethylene in stress-induced leaf ageing in salt- and drought-treated *A. halimus* remain unknown.

The present work was undertaken to compare the impacts of water stress and salinity on leaf senescence in relation to ABA and ethylene synthesis. Several criteria related to leaf senescence were considered (chlorophyll (Chl) concentration, reactive oxygen species content and lipid peroxidation index) and the nature of accumulated sugars as well as their contribution to osmotic adjustment of stressed plants was determined.

Materials and methods

Plant material and growth conditions

Seeds of *Atriplex halimus* were collected from the region of Monastir (Tunisia) (36° 13′ N; 10° 23′ W). After removal of the fruiting 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 and 20 °C during the night, under a PAR of 170 μmol m^{-2} s^{-1} and a photoperiod of 16 h. After 5 weeks, seedlings were distributed among tanks containing 2 L of nutrient solution containing (in mM) 5 KNO_3, 1 $NH_4H_2PO_4$, 0.5 $MgSO_4$, 5.5 $Ca(NO_3)_2$ and (in μM) 25 KCl, 10 H_3BO_3, 1 $MnSO_4$, 1 $ZnSO_4$, 0.25 $CuSO_4$, 10 Na_2MoO_4 and 50 mg L^{-1} FeEDTA. Solutions were renewed each week. Plants (8 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 days after transfer to nutrient solution. For salt stress, NaCl was added to the nutrient solution to obtain a final concentration of 160 mM (EC = 17.4 dS cm^{-1} and $\Psi s = -0.68$ MPa). For water stress, PEG (10,000; Sigma–Aldrich, Belgium) was added to the nutrient solution to reach a final dose of 15% (EC = 1.02 dS cm^{-1} and $\Psi s = -0.64$ MPa). Plants maintained in the absence of NaCl and PEG were used as controls.

In another set of experiments, plants were exposed under the same environmental conditions to similar stress factors in the presence or in the absence of 50 μM ABA (ABA, Sigma–Aldrich, Germany) added to nutrient solution. Treatments were applied over 10 days.

Plant growth and water relations

Plant growth was estimated by measuring the plant height and leaf number of 20 plants every 2 days. The number of leaves was determined by recording all the leaves on the main stem with blades longer than 1 cm. Plant growth was also determined on the basis of shoot dry weight (DW) per plant (estimated on 10 individual plants per treatment after 48 h of harvested tissues in an oven at 70 °C).

Shoot water potential (Ψ_w) and leaf osmotic potential (Ψ_s) were determined between 12:00 and 14:00. Shoot Ψ_w was evaluated immediately after sampling using the pressure chamber method. For Ψ_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 tissular sap was analyzed for Ψ_s estimation. Osmolarity (*c*) was assessed with a vapor pressure osmometer (Wescor 5500) and converted from mosmol kg^{-1} to MPa using the formula: Ψ_s (MPa) = $-c$(mosmol kg^{-1}) × 2.58 × 10^{-3} according to the Van't Hoff equation. To eliminate the effect of water loss on the possible changes in Ψs (which should not be regarded as osmotic adjustment *sensus stricto*), Ψ_s values were adjusted to the water content (WC) of unstressed tissues according to $X \times Y/Z$, where X is the measured Ψ_s and Y and Z are the WC of the stressed and unstressed tissues, respectively.

Osmocompatible solutes determination

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) using a spectrophotometer (Beckman DU-640). For glycinebetaine determination, collected leaves and roots (200 mg) were mixed with 5 mL 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 Na_2SO_4 in deionized water (pH adjusted to 3.7 with 1 N H_2SO_4) at a flow-rate of 0.8 mL min^{-1}. Detection was performed by a UV detector (Bio-Rad 1801 UV monitor) and quantification was performed by the ValueChrom_HPLC system (BioRad Chromatography Software version 4).

Total 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