REVIEW ARTICLE

The tolerance of *Atriplex halimus* L. to environmental stresses

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Abstract

Atriplex halimus L. (Amaranthaceae) (Mediterranean Saltbush) is a perennial, halophytic shrub that possesses the C4 photosynthetic anatomy and physiology. It grows under semi-arid and arid conditions (annual rainfall < 600 mm) from Macaronesia, through the Mediterranean basin countries and into western Asia, being particularly common on saline and degraded soils. Many studies have shed light on the physiological and biochemical mechanisms that, together with the morphological and anatomical features of this species, contribute to its notable tolerance of important abiotic stresses: salinity, drought, extreme temperatures and soil contamination by trace elements. These will be discussed here, highlighting their shared and distinct features. Certain processes are common to two or more stress responses: for example, vacuolar accumulation of sodium and the cytoplasmic accumulation of compatible osmolytes - part of the process of osmotic adjustment - are vital components of the adaptation to drought, salinity and cold. Others, such as oxalate accumulation upon trace elements exposure, seem to be stress-specific, while leaf surface vesiculated hairs (trichomes) and abscisic acid have distinct functions according to the stress. The relevance of these mechanisms to the use of *A. halimus* in soil remediation and as livestock forage is discussed.

Key words: Cold, Drought, Salinity, Stress, Trace elements

Introduction

Atriplex halimus L. (Amaranthaceae) is an upright perennial shrub. It has been planted for animal fodder and physical stabilisation of the soil throughout the arid and semi-arid zones of the Mediterranean basin and beyond. *A. halimus* occurs naturally throughout Eurasia, from Macaronesia, through the Mediterranean basin and into the Middle East and western Asia: including Portugal, France, Spain, Italy, Greece, Turkey, Cyprus, Israel, Syria, Lebanon, Jordan, Tunisia, Morocco, Algeria, Libya, Egypt and Saudi Arabia (Le Houérou, 1992; Al-Turki et al., 2000; Walker et al., 2005). It grows in zones of low annual rainfall (R)

and high potential evapotranspiration (PET): many of these can be classified as arid (R = 100-400 mm, R:PET = 0.06-0.28) or semi-arid (R = 400-600 mm, R:PET = 0.28-0.45). It is most common at sunny sites, on neutral or alkaline soils (pH 7.0–11.0) which are often saline (electrical conductivity of a saturated soil paste, EC_s > 4 dS m^{-1}) (Le Houérou, 1992).

Due to their morphological differences, Le Houérou (1992) divided *A. halimus* into two sub-species or varieties: *halimus* and *schweinfurthii* (Figure 1). For example, *halimus* has a more-erect habit and is smaller (0.5-2.0 m tall compared to 1.0-3.0 m for *schweinfurthii)*. Sub-species *schweinfurthii* grows in arid or saline zones (North Africa, the eastern Mediterranean basin, the Middle East and western Asia) and *halimus* in semi-arid, less-saline areas (in the western Mediterranean basin and Macaronesia) (Le Houérou, 1992; Walker et al., 2005). According to Walker et al. (2005), the sub-species *halimus* and *schweinfurthii* are diploid (2n=2x=18) and tetraploid (2n=4x=36), respectively.

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Figure 1. Plants of *Atriplex halimus*: sub-species *schweinfurthii* (left) and *halimus* (right). Total height of calibrated pole (in bush on the right) = 1.10 m.

Perennial plant species which are exposed to extreme conditions (heat, drought, salinity, high light intensity) in arid and semi-arid zones possess morphological, anatomical, biochemical and physiological adaptations. They includes members of the genera *Acacia, Artemisia, Atriplex, Colutea, Eucalyptus, Larrea, Limonium, Medicago, Prosopis, Salsola, Salicornia* and *Suaeda*, and of the Cactaceae (e.g., genus *Opuntia*) (Azam et al., 2012). This review is focused on *A. halimus* for several reasons. *Atriplex* constitutes a cosmopolitan genus of more than 400 species distributed over several continents (Le Houérou, 1992) and *A. halimus* can be considered a model species for this important genus. *Atriplex halimus* has a wide geographical distribution, is of economic importance and populations from sites of differing environmental conditions have been studied with regard to the traits responsible for their adaptation to environmental stresses, particularly salinity, drought, extreme temperatures and excess trace elements (TEs). However, the data obtained have not been considered as a whole. In the following sections, we review these findings and highlight both the mechanisms which are unique to a particular stress and those which are common to two or more.

Salinity

Although sodium (Na^+) is required as a micronutrient by C_4 species such as *A. halimus* (Brownell and Crossland, 1972), high levels of salinity can inhibit seed germination and plant growth through effects on water uptake and transport, due to the low water potential (Ψ_w) of saline media, the specific toxic effects of ions (generally Na^+ and Cl^-) accumulated in plant tissue and imbalances in the tissue levels of mineral nutrients (Munns, 2002). Le Houérou (1992) stated that *A. halimus* can withstand soil salinity equivalent to EC_s values of 25-30 dS m^{-1}. However, differences in salt tolerance among populations, according to the salinity of the soil in which they grow, have been reported.

The seed germination of *A. halimus* is more salt-tolerant than that of *A. canescens* (Pursh) Nutt. or *A. nummularia* Lindl. (Mâalen and Rahmoune, 2009). Debez et al. (2001) found the germination of a coastal population of *A. halimus* to be more tolerant to salinity than that of an inland population from a non-saline site (total inhibition at 350 and 700 mM NaCl, respectively), while Bajji et al. (2002) found that concentrations of NaCl $\geq$ 50 mM inhibited germination and perhaps transfer of mineral nutrients from the seed to the seedling. In both studies, the inhibition of germination was reversible (by removal of the external salt) and apparently due to an osmotic effect. Abbad et al. (2004) found that 85 mM NaCl was enough to inhibit significantly the germination of seven Moroccan populations of *A. halimus*; the reversibility of the inhibition was greater for a coastal population, indicating greater adaptation to saline soil. Autumn and winter rainfall leaches salt from the surface soil, as well as salt and saponins (which can inhibit *A. halimus* germination; Askham and Cornelius, 1971) from the bracteoles in the fruits, creating favourable conditions for seed germination.

The halophytic nature of this species has been demonstrated in nutrient solution studies. Moderate levels of NaCl (< 150-200 mM) added to a control nutrient solution stimulate growth while higher concentrations are inhibitory. Ben Hassine et al. (2008, 2009) found that, after 10 days, the shoot dry weight of a population from a saline site was stimulated at 40 and 160 mM NaCl, whereas that of a population from a non-saline, semi-arid site was stimulated at 40 mM but decreased at 160 mM NaCl. Nemat Alla et al. (2011) reported that increasing the NaCl concentration from the control level (no added NaCl) to 50 mM increased growth, after a 30-day exposure, while 300 or 550 mM NaCl produced no decrease in shoot growth. Ben Ahmed et al. (1996) found that, for plants grown from the seeds of a population from a coastal site, growth (dry mass) was optimal at 50-200 mM NaCl, but fell back to the control values (0 mM NaCl) at 300 mM.

The reasons for the enhanced growth of *A. halimus* at low external concentrations of salt have been investigated. Martínez et al. (2005) ascribed improved growth at 50 mM NaCl to possible Na^+ enhancement of photosynthesis and sugar synthesis and/or transport. Boughalleb et al. (2009) related enhanced growth to improvements in stomatal conductance, transpiration and CO_2 assimilation at moderate external NaCl levels (< 300 mM); Khedr et al. (2011) suggested that vacuolar Na^+ accumulation increases cell volume and the photoassimilatory surface area of the leaves, stimulating growth. Results indicating an adaptation of *A. halimus* glutamate dehydrogenase (EC 1.4.1.2) to high tissue NaCl levels were presented by Priebe and Jäger (1978). Sadder et al. (2013) demonstrated that salt-exposed plants had increased expression of specific genes: those controlling the synthesis of acetohydroxy acid reductoisomerase (EC 1.1.1.86), early-responsive dehydration stress-related proteins and ER-lumenal protein. Their expression was much higher at 150 mM than at 300 mM NaCl, indicating their specificity for growth-stimulating doses of salt.

Like other halophytes, *A. halimus* accumulates the ions responsible for salinity - Na^+ (Martínez et al., 2005; Nemat Alla et al., 2012) and Cl^- and other anions (Ben Ahmed et al., 1996; Ben Hassine et al., 2009) - in its tissues and is able to compartmentalise them in the vacuole due to efficient intracellular transport (Khedr et al., 2011). This osmotic adjustment (OA, the active intracellular accumulation of osmolytes) generates low values of tissue Ψ_w; even-lower coincident

values of osmotic potential (Ψ_s) (e.g., -7.5 MPa; Bajji et al., 1998) maintain tissue turgor. Hence, the plants can maintain water uptake from extremely-saline external media. Mozafar et al. (1970) found preferential uptake of Na^+, relative to potassium (K^+), even when K^+ was present at a higher concentration in the nutrient solution. Although exposure to salinity can diminish tissue K^+ concentrations, growth is unaffected or even stimulated (Bajji et al., 1998; Nemat Alla et al., 2011), reflecting the low total amount of K^+ required to fulfil its specific cytosolic roles and its substitution by other cations such as Na^+ in its role as a vacuolar osmolyte (Walker et al., 1996).

An important anatomical feature of *A. halimus* (and other *Atriplex* spp.) in relation to salt tolerance are the vesiculated hairs (Mozafar and Goodin, 1970) or vesicular trichomes (Smaoui et al., 2011) present on the leaf surface. These living cells consist of balloon-like hairs or bladder cells (Smaoui et al., 2011), 80-200 μm in diameter, attached to a stalk that is embedded in an epidermal cell. They act as a sink for salt as external salinity increases (Mozafar and Goodin, 1970); salt excretion into them from neighbouring mesophyll and epidermal cells can occur symplastically, apoplastically or via organelles (Smaoui et al., 2011). Ben Hassine et al. (2009) showed that - relative to a population from a non-saline site - a population from a saline site excreted a greater percentage of its Na^+ and Cl^- into the vesiculated hairs, to protect the photosynthetic tissues, and was better able to maintain its tissue K^+ concentration under high external salinity.

Simultaneous to the accumulation of ionic osmolytes in the vacuole, there is cytosolic and organellar accumulation of "compatible" organic osmolytes, which do not affect metabolic processes even at very-high concentrations, in order to maintain the osmotic balance across the tonoplast, protect membranes, organelles and proteins and (in the case of proline) to regulate metabolism, act as anti-oxidants and promote recovery once the stress has eased (Szabados and Savouré, 2009). Bajji et al. (1998) reported that increasing external salinity (150-600 mM NaCl) provoked accumulation of soluble sugars and proline in leaves but not in roots, while it had no clear effect on the accumulation of quaternary ammonium compounds (QACs). For the same population (Kairouan, Tunisia), Martínez et al. (2005) found that a growth-promoting concentration of NaCl (50 mM) raised root and leaf proline but did not affect leaf concentrations of soluble sugars or the QAC glycinebetaine (GB).

Ben Hassine et al. (2008, 2009) found that a more-tolerant Tunisian population, from a saline site, accumulated higher levels of Na^+ and Cl^- and had lower values of shoot Ψ_w and higher stomatal conductivity than a less-tolerant population. The authors suggested that its higher GB accumulation, in the chloroplasts, may have been responsible for its maintenance of photosystem II activity. The findings of Bouchenak et al. (2012) also suggest roles for QACs and proline in the salt tolerance of this species.

At elevated external salt concentrations (≥ 300 mM NaCl), the protective mechanisms of *A. halimus* are not sufficient to maintain stomatal conductance (Nemat Alla et al., 2011), the root plasma membrane permeability, root hydraulic conductivity and chlorophyll content (Nedjimi and Daoud, 2009) or the photosynthetic machinery (Schwarz and Gale, 1981; Boughalleb et al., 2009; Khedr et al., 2011), or to protect the integrity of intracellular organelles (Blumenthal-Goldschmidt and Poljakoff-Mayber, 1968; Kelley, 1974; Wong and Jäger, 1978). Very-high external salinity also diminishes tissue levels of mineral nutrients such as inorganic phosphorus, Mg^{2+} and Ca^{2+} (Bajji et al., 1998). This physiological and metabolic disruption stimulates the cellular anti-oxidant systems. Bouchenak et al. (2012) found that an Algerian population from a saline site, which exhibited greater tolerance of 300 mM NaCl than one from a non-saline site, had greater activity of certain anti-oxidant enzymes: catalase (EC 1.11.1.6) (which catalyses the breakdown of hydrogen peroxide into water and oxygen), peroxiredoxins (EC 1.11.1.15) (involved in the scavenging of organic peroxides) and peptide methionine sulphoxide reductases (EC 1.8.4.6) (which have a role in the repair of oxidised proteins). Schwarz and Gale (1981) described an increase in the maintenance respiration of *A. halimus* with increasing external salinity and considered that, while this is an adaptive mechanism, it contributes to growth decline at and above the toxicity threshold of salinity.

Drought

As shown by Rawson and Begg (1977), xerophytic C_4 species such as *A. halimus* are efficient in their water use, since a lesser degree of stomatal opening is needed for maximal activity of phosphoenol pyruvate (PEP) carboxylase (EC 4.1.1.31), relative to ribulose-1,5-bisphosphate carboxylase oxygenase (EC 4.1.1.39), the CO_2-incorporating enzyme of the C_3 photosynthetic pathway. *Atriplex halimus* develops a dual root system: long, main roots and secondary roots borne on them permit the uptake of water from depths of up to 5 m, while fine, shoot-borne roots can access moisture and nutrients following rainfall (Guerrero-Campo et al., 2006). Despite its adaptation to semi-arid and arid environments, severe water deprivation (in pot or hydroponic studies) produces growth inhibition in *A. halimus*, a decline in tissue water content and general decreases in leaf number and area (to minimise water loss), height and ramification (Martínez et al., 2003, 2004; Essafi et al., 2006; Nedjimi, 2012).

Water-deprived plants of *A. halimus* generate very-low values of Ψ_w and Ψ_s (as low as –4.20 and -6.57 MPa, respectively; Bajji et al., 1998). Differences between populations of *A. halimus*, related to the pedo-climatic conditions at their native sites, have been found with respect to their mechanism of adaptation to external water shortage and their tolerance (maintenance of growth). Martínez et al. (2003, 2004) compared populations from a saline, coastal site in Morocco and a dry, inland site in Tunisia. Their common physiological responses to water deprivation were declines in shoot Ψ_w and Ψ_s, leaf relative water content (RWC), transpiration and CO_2 assimilation, and increases in leaf K^+ and Na^+. Also, while leaf proline levels were unaffected for both populations, the concentrations of GB and sugars increased. Although the OA of populations from more-arid sites was greater when exposed to polyethylene glycol (PEG) in order to lower the external Ψ_w (Martínez et al., 2004; Ben Hassine et al., 2008), tolerance was not associated strongly with this trait, perhaps due to the energetic costs (Martínez et al., 2004). These authors concluded that improved water use efficiency, perhaps through abscisic acid (ABA)-mediated regulation of stomatal conductance (Ben Hassine et al., 2009), contributed to the maintenance of growth under restricted water supply. Ben Hassine et al. (2010) also played down the role of OA in drought tolerance of *A. halimus*. Martínez et al. (2005) found that PEG exposure raised the root and leaf levels of Na^+ and the leaf levels of GB and soluble sugars, but did not affect proline. Ben Hassine and co-workers (Ben Hassine et al., 2008, 2009; Ben Hassine and Lutts, 2010) studied the effects of water deprivation on two Tunisian populations of *A. halimus*, one from an arid site and one from a saline site. The former was more tolerant and accumulated more proline, had more-negative values of Ψ_w and Ψ_s (and higher turgor), a higher CO_2 assimilation rate and lower stomatal conductance (and hence higher water use efficiency). A role of Na^+ in drought tolerance was

indicated by the results of Ben Hassine et al. (2010) and Nedjimi (2012).

Stress-specific responses to drought and salinity

Comparisons of the physiological and biochemical responses of plants of the same population of *A. halimus* to salinity and water deprivation have demonstrated the involvement of both common and distinct mechanisms.

The results of Ben Hassine et al. (2009) indicate a role for the polyamines spermine and spermidine in the response to salinity (for salt-adapted populations) and for putrescine in drought tolerance of populations from arid sites. These authors proposed that the role of ABA differs between the two stresses: under osmotic stress, it enhances stomatal regulation and water use efficiency whereas under external salinity it promotes excretion of Na^+ and Cl^- into the vesiculated hairs. They also suggested that spermine and spermidine are involved in the excretion process and that ABA stimulates their synthesis and conversion into active forms. Ben Hassine and Lutts (2010) found that salt-exposed plants (160 mM NaCl) showed greater accumulation of ABA, GB and sucrose than water-deprived plants (15% PEG), which accumulated more glucose, fructose and ethylene; the latter was associated with premature senescence, manifested as the accumulation of reactive oxygen species (ROS), membrane peroxidation and chlorophyll degradation. Ben Hassine and co-workers (Ben Hassine et al., 2008, 2009; Ben Hassine and Lutts, 2010) suggested that the slower (chloroplastic) accumulation of GB protects the photosynthetic apparatus against permanent soil salinity, whilst proline (which accumulates more quickly) may protect against oxidative damage and also regulate carbon and nitrogen metabolism when the plants are faced with short-term drought.

Khedr et al. (2012) studied the Dehydration Responsive Element Binding (DREB) transcription factor in *A. halimus*, which regulates the expression of many stress-inducible genes, and showed that it was up-regulated greatly by both water deprivation and salinity; in the latter case, it was the osmotic component of the stress that was responsible. Nemat Alla et al. (2012) published a metabolomics-based analysis of the changes in *A. halimus* caused by exposure to salt (NaCl) or water deprivation (PEG). They found common responses to these two stresses (e.g., up-regulation of the tricarboxylic acid cycle and synthesis of β-alanine) and others that were specific to salinity (e.g., up-regulation of ABA transport and alkaloid synthesis) or PEG (e.g.,

up-regulation of tryptophane metabolism). Overall, salinity produced more metabolic disturbance than water deprivation.

It must be borne in mind that in their natural environment, plants are often exposed simultaneously to drought and salinity. Nedjimi (2012) reported that, in an Algerian *chott* soil, a decline in soil moisture from 20.1% in autumn to 7.7% the following summer was accompanied by increases in soil EC_s (from 2.0 to 4.5 dS m^{-1}) and water-extractable Na^+ and K^+ (from 34.5 to 66.6 mM and from 4.1 to 34.7 mM, respectively).

Extreme temperatures and light

Atriplex halimus is adapted to climate zones having summers with very-high light intensity and temperature. Since it possesses the C_4 carboxylation pathway, the optimum temperature for photosynthesis is relatively high, around 35ºC (Shomer-Ilan et al., 1981; Le Houérou, 1992; Zervoudakis et al., 1998). Streb et al. (1997), studying leaves of *A. halimus* plants exposed to photosynthetically active radiation in the field as high as 2200 μmol m^{-2} s^{-1}, found a limited capacity for the dissipation of light energy and for scavenging of ROS. They concluded that *A. halimus* is able to withstand high light intensity due to mutual shading of leaves and reflection of light from the leaf surface by a layer of salt crystals formed after the collapse of vesiculated hairs (Mozafar and Goodin, 1970), which gives the leaves a greenish-grey colour (Figure 1).

Le Houérou (1992) placed *A. halimus* in a group of *Atriplex* species exhibiting moderate cold tolerance (able to withstand temperatures as low as -12ºC). Walker et al. (2008) found that the cold tolerance of *A. halimus* leaves was greater in early winter (acclimation) than in spring (following de-acclimation). In this study, plant tolerance of temperatures in the field as low as -18ºC correlated positively with leaf accumulation of Na^+, K^+, amino acids, QACs and soluble sugars, the leaf sap Ψ_w and the % dry matter (DM) of the tissue. Mirroring the behaviour shown in drought and salinity adaptation, the leaf Ψ_w and Ψ_s values of cold-acclimated plants in winter (as low as -2.69 and -6.32 MPa, respectively) were lower than in the following spring. This underlines the role of OA with regard to minimising cellular dehydration through water loss to extracellular ice, the main cause of freezing damage (Xin and Browse, 2000); it also ensures water uptake from very-cold soils (having low matric potentials). Walker et al. (2008) found diploid populations (sub-species *halimus*) to be more cold-tolerant than tetraploid ones

(*schweinfurthii)*, probably due to the less-negative Ψ_s values of the latter. Support for a role of OA in cold tolerance was provided by Aouissat et al. (2009), who demonstrated that the tolerance of *A. halimus* was improved by salinisation of the soil with NaCl, which raised leaf Na^+ (and soluble sugar) concentrations. Compatible organic osmolytes may help to protect membrane and protein structure, hence acting as "osmoprotectants" in the face of freezing temperatures. For example, Salahas et al. (2002) found that high concentrations of GB and proline protected PEP carboxylase and pyruvate orthophosphate dikinase (EC 2.7.9.1) against cold inactivation.

Trace elements

Trace elements such as arsenic (As), copper (Cu), cadmium (Cd), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) are important contaminants of soil due to previous and/or ongoing anthropogenic activities such as agriculture, industry and mining. Since *A. halimus* has colonised TEs-contaminated sites, for example in southern Spain (Lutts et al., 2004; Márquez-García et al., 2013) and Algeria (Lotmani et al., 2011), the processes which may contribute to this have been investigated.

The germination of *A. halimus* was found to be high in TEs-contaminated soils (Lotmani et al., 2011; Martínez-Fernández and Walker, 2012), while Márquez-García et al. (2013) found that the speed and final percentage of germination were unaffected by Cu, Mn, Ni or Zn concentrations in solution as high as 2 mM. Experiments performed with *A. halimus* in pots of contaminated soil (Manousaki and Kalogerakis, 2009; Martínez-Fernández and Walker, 2012; Pérez-Esteban et al., 2013), in hydroponic culture (Lefèvre et al., 2009; Lutts et al., 2004) or in Petri dishes (Márquez-García et al., 2013) show that its growth is tolerant of high concentrations of Cd, Cu, Mn, Ni, Pb or Zn in the medium.

Lutts et al. (2004) found that a 3-week exposure to elevated external Cd, whch produced leaf DM Cd concentrations as high as 618 μg g^{-1}, did not reduce shoot growth significantly, for plants of *A. halimus* grown from seeds of a population from a contaminated site in southern Spain. This indicates a very-high degree of tolerance, since values in excess of 5 μg g^{-1} are generally phytotoxic (Kabata-Pendias, 2001). The Cd tolerance mechanisms may include precipitation with oxalate in the stems (Lutts et al., 2004) and excretion into vesiculated hairs (Lefèvre et al., 2009). In saline nutrient solutions (containing high levels of KCl or NaCl), Cd tolerance is greater and coincides with decreased Cd uptake - possibly resulting from the formation of Cd-Cl complexes which are taken up to a lesser extent than Cd^{2+} (Lefèvre et al., 2009). This is important, since contaminated soils at former mine sites are often saline (Clemente et al., 2012). Lefèvre et al. (2009) found no effect of Cd on soluble sugars accumulation, but did find accumulation of GB, proline, spermine and spermidine, as with salt exposure (Ben Hassine et al., 2009). Nedjimi (2009) found that high external concentrations of calcium inhibit Cd uptake by *A. halimus*, suggesting that tolerance might be greater on calcareous soils. Manousaki and Kalogerakis (2009) showed that *A. halimus* is efficient at excluding Pb from its leaves, although most species exhibit such behaviour since Pb is a highly-immobile element which tends to remain in the soil and roots rather than be transported to the shoot. *Atriplex halimus* has a high tolerance of elevated external and internal Zn concentrations, which may result from co-precipitation with oxalate (Lutts et al., 2004). Tapia et al. (2013) demonstrated that *A. halimus* is able to cope with high tissue levels of As (up to 5.5 μg g^{-1} fresh leaf) and that it may be suitable for phytomanagement of As-contaminated areas.

Common and distinct mechanisms of abiotic stress tolerance

From the preceding text, it can be seen that the responses of *A. halimus* to external salinity, drought (water deprivation) and sub-zero temperatures, all of which can provoke cellular damage through uncontrolled tissue dehydration, share the process of OA, although its extent and importance differ among stresses and populations. Among the ionic osmolytes, Na^+ seems to accumulate preferentially. These adjustments allow the plant to maintain water uptake from external media of low Ψ_w (salinity, drought) or, in the case of cold tolerance, to minimise leaf dehydration. High concentrations of TEs external to the roots can also perturb plant water relations (Barceló and Poschenrieder, 1990), producing similar responses in *A. halimus*. Lefèvre et al. (2010) showed commonality in the response of *A. halimus* cell lines to PEG-induced dehydration and Cd, regarding the production of ROS. A PEG-resistant cell line exhibited higher growth in the presence of Cd than a sensitive line, although Cd accumulation was higher in the former, as well as greater ability to cope with oxidative stress in relation to an increase of endogenous antioxidants (glutathione and ascorbic

acid), a high constitutive activity of superoxide dismutase (EC 1.15.1.1) and a Cd-induced increase in glutathione reductase (EC 1.6.4.2). The vesiculated hairs seem to have roles in both salt and Cd tolerance, acting as sites of deposition in order to protect the cytoplasm and photosynthetic apparatus. The function of ABA apparently differs between salinity exposure (stimulating Na^+ and Cl^- excretion into the vesiculated hairs) and drought (effecting water loss reduction), whereas oxalate accumulation seem to be stress-specific, as a mechanism of TEs detoxification. The characteristics of *A. halimus* which enable it to withstand extreme environmental conditions are listed in Table 1.

As for many species, physiological studies on *A. halimus* under field conditions are lacking. This is important because field-grown plants subjected to drought and/or salinity and/or high soil levels of TEs are usually exposed simultaneously to temperatures and light intensities much higher than in controlled conditions, which influence their anatomy, morphology, physiology and biochemistry.

Significance of stress tolerance mechanisms for the utilisation of *A. halimus*

The features which enable *A. halimus* to withstand extreme environmental conditions may influence its use. For example, the ability of *A. halimus* to stabilise soils physically, together with its high tolerance of TEs, could be exploited in the phytoremediation of TEs-contaminated (semi-)arid sites. Clemente et al. (2012) found that *A. halimus* formed a dense stand on a highly-contaminated site in southern Spain; however, its relatively-high levels of foliar Cd and Zn were a concern, since they could lead to dissemination of the contaminants through leaf litter or feeding by herbivores. The often-high salt contents of *A. halimus* shoot tissue (up to 10% Na^+ in DM; Bhattacharya, 1989; Walker et al., 2008) require an elevated intake of water by livestock (Mirreh et al., 2000), while its levels of oxalate (up to 10% of DM) may reduce the palatability (Abu-Zanat et al., 2003). However, its accumulation of GB may have beneficial effects on monogastric animals such as pigs and poultry, due to its roles as a methyl group donor, in protein and energy metabolism, as an osmoprotectant of intestinal cells and gut microbes and in the improved digestibility of fibre (Ratriyanto et al., 2009).

Table 1. The characteristics of *A. halimus* which confer adaptation to or tolerance of environmental extremes.

Characteristic	Environmental extreme
Deep root system	Drought
Mutual shading of leaves	High light intensity
C_4 photosynthesis	High temperature, drought
High water use efficiency	Drought
Salt accumulation	Salinity, drought, cold
Osmotic adjustment	Salinity, cold, drought
Accumulation of "compatible" organic osmolytes	Drought, cold, salinity
Deposition of excess ions in vesiculated hairs	Salinity, trace elements
Salt release from vesiculated hairs	High light intensity
Accumulation of oxalate	Trace elements

References

Abbad, A., A. El Hadrami and A. Benchaabane. 2004. Germination responses of the Mediterranean saltbush (*Atriplex halimus* L.) to NaCl treatment. J. Agron. 3:111-114.

Abu-Zanat, M. W, F. M. Al-Hassanat, M. Alawi and G. B. Ruyle. 2003. Oxalate and tannins assessment in *Atriplex halimus* L. and *A. nummularia* L. J. Range Manage. 56:370-374.

Al-Turki, T. A., S. Omer and A. Ghafoor. 2000. A synopsis of the genus *Atriplex* L. (Chenopodiaceae) in Saudi Arabia. Feddes Repert. 111:261-293.

Aouissat, M., D. J. Walker, M. Belkhodja, S. Fares and E. Correal. 2009. Freezing tolerance in Algerian populations of *Atriplex halimus* and *Atriplex canescens*. Span. J. Agric. Res. 7:672-679.

Askham, L. R. and D. R. Cornelius. 1971. Influence of desert saltbush saponin on germination. J. Range Manage. 24:439-4.

Azam, G., C. D. Grant, I. K. Nuberg, R. S. Murray and R. K. Misra. 2012. Establishing woody perennials on hostile soils in arid and semi-arid regions – a review. Plant Soil 360:55-76.

Bajji, M., J.-M. Kinet and S. Lutts. 1998. Salt stress effects on roots and leaves of *Atriplex halimus* L. and their corresponding callus cultures. Plant Sci. 137:131-142.

Bajji, M., J.-M. Kinet and S. Lutts. 2002. Osmotic and ionic effects of NaCl on germination,

early seedling growth and ion content of *Atriplex halimus* (Chenopodiaceae). Can. J. Bot. 80:297-304.

Barceló, J. and C. Poschenrieder. 1990. Plant water relations as affected by heavy metal stress: a review. J. Plant Nutr. 13:1-37.

Ben Ahmed, H., E. Zid, M. El Gazzah and C. Grignon. 1996. Croissance et accumulation ionique chez *Atriplex halimus* L. Cah. Agric. 5:367-372.

Ben Hassine, H., M. E. Ghanem, S. Bouzid and S. Lutts. 2008. An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. J. Exp. Bot. 59:1315-1326.

Ben Hassine, H., M. E. Ghanem, S. Bouzid and S. Lutts. 2009. Abscisic acid has contrasting effects on salt excretion and polyamine concentrations of an inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus*. Ann. Bot. 104:925-936.

Ben Hassine, H., S. Bouzid and S. Lutts. 2010. Does habitat of *Atriplex halimus* L. affect plant strategy for osmotic adjustment? Acta Physiol. Plant. 32:325-331.

Ben Hassine, H. and S. Lutts. 2010. Differential responses of saltbush *Atriplex halimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene. J. Plant Physiol. 167:1448-1456.

Bhattacharya, A. N. 1989. Nutrient utilization of acacia, haloxylon, and atriplex species by Najdi sheep. J. Range Manage. 42:28-31

Blumenthal-Goldschmidt, S. and A. Poljakoff-Mayber. 1968. Effect of substrate salinity on growth and on submicroscopic structure of leaf cells of *Atriplex halimus* L. Aust. J. Bot. 16:469-478.

Bouchenak, F., P. Henri, F.-Z. Benrebiha and P. Rey. 2012. Differential resonses to salinity of two *Atriplex halimus* populations in relation to organic solutes and antioxidant systems involving thiol reductases. J. Plant Physiol. 169:1445-1453.

Boughalleb, F., M. Denden and B. Ben Tiba. 2009. Photosystem II photochemistry and physiological parameters of three fodder shrubs, *Nitraria retusa, Atriplex halimus* and *Medicago arborea* under salt stress. Acta Physiol. Plant. 31:463-476.

Brownell, P. F. and C. J. Crossland. 1972. The requirement for sodium as a micronutrient by species having the C_4 dicarboxylic photosynthetic pathway. Plant Physiol. 49:794-797.

Clemente, R., D. J. Walker, T. Pardo, D. Martínez-Fernández and M. P. Bernal. 2012. The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions. J. Haz. Mater. 223-224:63-71.

Debez, A., W. Chaibi and S. Bouzid. 2001. Effect of NaCl and growth regulators on germination of *Atriplex halimus* L. Cah. Agric. 10:135-138.

Essafi, N. E., M. Mounsif, A. Abousalim, M. Bendaou, A. Rachidai and F. Gaboune. 2006. Impact of water stress on the fodder value of *Atriplex halimus* L. New Zeal. J. Agric. Res. 49:321-329.

Guerrero-Campo, J., S. Palacio, C. Pérez-Rontomé and G. Montserrat-Martí. 2006. Effect of root system morphology on root-sprouting and shoot-rooting abilities in 123 plant species from eroded lands in north-east Spain. Ann. Bot.-London. 98:439-447.

Kabata-Pendias, A. 2001. Trace Elements in Soils and Plants. 3rd Ed. CRC Press LLC, Boca Raton, FL.

Kelley, D. B. 1974. Salinity effects on growth and fine structure of *Atriplex halimus* L. Masters dissertation, Texas Tech University, Houston.

Khedr, A. H. A., M. S. Serag, M. M. Nemat-Alla, A. Z. Abo El-Naga, R. M. Nada, W. P. Quick and G. M. Abogadallah. 2011. Growth stimulation and inhibition by salt in relation to Na^+ manipulating genes in xero-halophyte *Atriplex halimus* L. Acta Physiol. Plant. 33:1769-1784.

Khedr, A. H. A., M. S. Serag, M. M. Nemat-Alla, A. Z. Abo El-Naga, R. M. Nada, W. P. Quick and G. M. Abogadallah. 2012. A *DREB* gene from the xero-halophyte *Atriplex halimus* is induced by osmotic but not ionic stress and shows distinct differences from glycophytic homologues. Plant Cell Tiss. Organ Cult. 106:191-206.

Lefèvre, I., G. Marchal, P. Meerts, E. Correal and S. Lutts. 2009. Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex halimus* L. Environ. Exp. Bot. 65:142-152.

Lefèvre, I., G. Marchal, M. E. Ghanem, E. Correal and S. Lutts. 2010. Cadmium has contrasting effects on polyethylene glyol-sensitive and resistant cell lines in the Mediterranean halophyte *Atriplex halimus* L. J. Plant Physiol. 167:365-374.

Le Houérou, H. H. 1992. The role of saltbushes (*Atriplex* spp.) in arid land rehabilitation in the Mediterranean Basin: a review. Agroforest. Syst. 18:107-148.

Lotmani, B., L. Fatarna, A. Berkani, J. Rabier, P. Prudent and I. Laffont-Schwob. 2011. Algerian populations of the Mediterranean saltbush, *Atriplex halimus*, tolerant to high concentrations of lead, zinc, and copper for phytostabilization of heavy metal-contaminated soils. Eur. J. Plant Sci. Biotechnol. 5:20-26.

Lutts, S., I. Lefèvre, S. Delpérée, S. Kivits, C. Dechamps, A. Robledo and E. Correal. 2004. Heavy metal accumulation by the halophyte species Mediterranean saltbush. J. Environ. Qual. 33:1271-1279.

Manousaki, E. and N. Kalogerakis. 2009. Phytoextraction of Pb and Cd by the Mediterranean saltbush (*Atriplex halimus* L.): metal uptake in relation to salinity. Environ. Sci. Pollut. Res. 16:844-854.

Martínez, J. P., J. F. Ledent, M. Bajji, J. M. Kinet and S. Lutts. 2003. Effect of water stress on growth, Na$^+$ and K$^+$ accumulation and water use efficiency in relation to osmotic adjustment in two populations of *Atripex halimus* L. Plant Growth Regul. 41:63-73.

Martínez, J. P., S. Lutts, A. Schank, M. Bajji and J. M. Kinet. 2004. Is osmotic adjustment required for water stress resistance in the Mediterranean shrub *Atripex halimus* L? J. Plant Physiol. 161:1041-1051.

Martínez, J.-P., J.-M. Kinet, M. Bajji and S. Lutts. 2005. NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L. J. Exp. Bot. 56:2421-2431.

Martínez-Fernández, D. and D. J. Walker. 2012. The effects of soil amendments on the growth of *Atriplex halimus* and *Bituminaria bituminosa* in heavy metal-contaminated soils. Water, Air, Soil Pollut. 223:63-72.

Márquez-García, B., C. Márquez, I. Sanjosé, F. J. J. Nieva, P. Rodríguez-Rubio and A. F. Muñoz-Rodríguez. 2013. The effects of heavy metals on germination and seedling characteristics in two halophyte species in Mediterranean marshes. Mar. Pollut. Bull. 70:119-124.

Mâalen, S. and C. Rahmoune. 2009. Toxicity of the salt and pericarp inhibition on the germination of some *Atriplex* species. Am. Eurasian J. Toxicol. Sci. 1:43-49.

Mirreh, M. M., A. A. Osman, M. D. Ismail, M.S. Al Daraan and M. M. Al Rowaili. 2000. Evaluation of six halophytic shrubs under center-pivot sprinkler irrigation. In: G. Gintburger, M. Bounejmate and A. Nefzaoui (Eds). pp. 293-308. Fodder Shrub Development in Arid and Semi-Arid Zones. ICARDA. Aleppo. Syria.

Mozafar, A. and J. R. Goodin. 1970. Vesiculated hairs: a mechanism for salt tolerance in *Atriplex halimus* L. Plant Physiol. 45:62-65.

Mozafar, A., J. R. Goodin and J. J. Oertli. 1970. Sodium and potassium interactions in increasing the salt tolerance of *Atriplex halimus* L.: II. Na$^+$ and K$^+$ uptake characteristics. Agron. J. 62:481-484.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25:239-250.

Nedjimi, B. 2009. Can calcium protect *Atriplex halimus* subsp. *schweinfurthii* against cadmium toxicity? Acta Bot. Gallica 156:391-397.

Nedjimi, B. 2012. Seasonal variation in productivity, water relations and ion contents of *Atriplex halimus* spp. *schweinfurthii* grown in Chott *Zehrez* wetland, Algeria. J. Saudi Soc. Agric. Sci. 11:43-49.

Nedjimi, B. and Y. Daoud. 2009. Effects of calcium chloride on growth, membrane permeability and root hydraulic conductivity in two *Atriplex* species grown at high (sodium chloride) salinity. J. Plant Nutr. 32:1818-1830.

Nemat Alla, M. M., A.-H. A. Khedr, M. M. Serag, A. Z. Abu-Alnaga and R. M. Nada. 2011. Physiological aspects of tolerance in *Atriplex*

halimus L. to NaCl and drought. Acta Physiol. Plant. 33:547-557.

Nemat Alla, M. M., A.-H. A. Khedr, M. M. Serag, A. Z. Abu-Alnaga and R. M. Nada. 2012. Regulation of metabolomics in *Atriplex halimus* growth under salt and drought stress. Plant Growth Regul. 67:281-304.

Pérez-Esteban, J., C. Escolástico, J. Ruiz-Fernández, A. Masaguer and A. Moliner. 2013. Bioavailability and extraction of heavy metals from contaminated soil by *Atriplex halimus*. Environ. Exp. Bot. 88:53-59.

Priebe, A. and H.-J. Jäger. 1978. Responses of amino acid metabolizing enzymes from plants differing in salt tolerance to NaCl. Oecologia 36:307-315.

Ratriyanto, A., R. Mosenthin, E. Bauer and M. Eklund. 2009. Metabolic, osmoregulatory and nutritional functions of betaine in monogastric animals. Asian-Aust. J. Anim. Sci. 22:1461-1476.

Rawson, H. M. and J. E. Begg. 1977. The effect of atmospheric humidity on photosynthesis, transpiration and water use efficiency of leaves of several plant species. Planta 134:5-10.

Sadder, M. T., F. Anwar and A. A. Al-Doss. 2013. Gene expression and physiological analysis of *Atriplex halimus* (L.) under salt stress. Austr. J. Crop Sci. 7:112-118.

Salahas, G., E. Corms and G. Zervoudakis. 2002. Cold inactivation of phosphoenolpyruvate carboxylase and pyruvate orthophosphate dikinase from the C4 perennial plant Atriplex halimus. Russ. J. Plant Physiol. 49:211-215.

Schwarz, M. and J. Gale. 1981. Maintenance respiration and carbon balance of plants at low levels of sodium chloride salinity. J. Exp. Bot. 32:933-941.

Shomer-Ilan, A., A. Nissenbaum and Y. Waisel. 1981. Photosynthetic pathways and the ecological distribution of the Chenopodiaceae in Israel. Oecologia 48:244-248.

Smaoui, A., Z. Barhoumi, M. Rabhi and C. Abdelly. 2011. Localization of potential ion transport pathways in vesicular trichome cells of *Atriplex halimus* L. Protoplasma 248:363-372.

Streb, P., E. Tel-Or and J. Feierabend. 1997. Light stress effects and antioxidative protection in two desert plants. Funct. Ecol. 11:416-424.

Szabados, L. and A. Savouré. 2009. Proline: a multifunctional amino acid. Trends Plant Sci. 15:89-97.

Tapia, Y., O. Diaz, C. Pizarro, R. Segura, M. Vines, G. Zúñiga and E. Moreno-Jiménez. 2013. *Atriplex atacamensis* and *Atriplex halimus* resist As contamination in Pre-Andean soils (northern Chile). Sci. Tot. Environ. 450/451:188-196.

Walker, D. J., R. A. Leigh and A. J. Miller. 1996. Potassium homeostasis in vacuolated plant cells. P. Natl. Acad. Sci. USA. 93:10510-10514.

Walker, D. J., I. Moñino, E. González, N. Frayssinet and E. Correal. 2005. Determination of ploidy and nuclear DNA content in populations of *Atriplex halimus* (Chenopodiaceae). Bot. J. Linn. Soc. 147:441-448.

Walker, D. J., P. Romero, A. de Hoyos and E. Correal. 2008. Seasonal changes in cold tolerance, water relations and accumulation of cations and compatible solutes in *Atriplex halimus* L. Environ. Exp. Bot. 64:217-224.

Wong, C. H. and H.-J. Jäger. 1978. Salt-induced vesiculation in mesophyll cells of *atriplex* species. Plant Sci. Lett. 12:63-68.

Xin, Z. and J. Browse. 2000. Cold comfort farm: the acclimation of plants to freezing temperatures. Plant Cell Environ. 23:893-902.

Zervoudakis, G., K. Angelopoulos, G. Salahas and C. D. Georgiou. 1998. Differences in cold inactivation of phosphoenolpyruvate carboxylase among C4 species: the effect of pH and of enzyme concentration. Photosynthetica 35:169-175.