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, ECs > 4 dS m⁻¹) (Le Houérou, 1992).

Due to their morphological differences, Le Houérou (1992) divided *A. halimus* into two subspecies 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.
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 (\(\Psi_w\)) 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. (Maalen 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. Martinez et al. (2005) ascribed improved growth at 50 mM NaCl to possible Na⁺-enhancement of photosynthesis and sugar synthesis and/or transport. Bouhalleb et al. (2009) related enhanced growth to improvements in stomatal conductance, transpiration and CO₂ 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 dehydrogenation stress-related proteins and ER-luminal 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⁺ (Martinez 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 Ψₑ; even-lower coincident values of osmotic potential (Ψₓ) (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 fulfi 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), Martinez 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 Ψᵰ 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, Mg²⁺ and Ca²⁺ (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 C₄ 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 CO₂-incorporating enzyme of the C₃ 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 Ψᵰ and Ψₛ (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 Ψᵰ and Ψₛ, leaf relative water content (RWC), transpiration and CO₂ 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 Ψᵰ (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 Ψᵰ and Ψₛ (and higher turgor), a higher CO₂ assimilation rate and lower stomatal conductivity (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 co-workers (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 ECₑ (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 C₄ carboxylation pathway, the optimum temperature for photosynthesis is relatively high, around 35°C (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 -12°C). 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 -18°C correlated positively with leaf accumulation of Na⁺, K⁺, amino acids, QACs and soluble sugars, the leaf sap Ψ₉ and the % dry matter (DM) of the tissue. Mirroring the behaviour shown in drought and salinity adaptation, the leaf Ψ₉ and Ψₛ 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 $\Psi_s$ 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-Garcia 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, which produced leaf DM Cd concentrations as high as 618 $\mu$g 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 $\mu$g 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 $\mu$g 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 $\Psi_w$ (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.

<table>
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<tr>
<th>Characteristic</th>
<th>Environmental extreme</th>
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<tr>
<td>Deep root system</td>
<td>Drought</td>
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<td>Mutual shading of leaves</td>
<td>High light intensity</td>
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<td>C₄ photosynthesis</td>
<td>High temperature, drought</td>
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<tr>
<td>High water use efficiency</td>
<td>Drought</td>
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<tr>
<td>Salt accumulation</td>
<td>Salinity, drought, cold</td>
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<td>Osmotic adjustment</td>
<td>Salinity, cold, drought</td>
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<td>Accumulation of “compatible” organic osmolytes</td>
<td>Drought, cold, salinity</td>
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<td>Deposition of excess ions in vesiculated hairs</td>
<td>Salinity, trace elements</td>
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<td>Salt release from vesiculated hairs</td>
<td>High light intensity</td>
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<td>Accumulation of oxalate</td>
<td>Trace elements</td>
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### References


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


