Plant salt tolerance: adaptations in halophytes

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BACKGROUND
Most of the water on Earth is seawater, each kilogram of which contains about 35 g of salts, and yet most plants cannot grow in this solution; less than 0.2% of species can develop and reproduce with repeated exposure to seawater. These ‘extremophiles’ are called halophytes.

SCOPE
Improved knowledge of halophytes is of importance to understanding our natural world and to enable the use of some of these fascinating plants in land re-vegetation, as forages for livestock, and to develop salt-tolerant crops. In this Preface to a Special Issue on halophytes and saline adaptations, the evolution of salt tolerance in halophytes, their life-history traits and progress in understanding the molecular, biochemical and physiological mechanisms contributing to salt tolerance are summarized. In particular, cellular processes that underpin the ability of halophytes to tolerate high tissue concentrations of Na⁺ and Cl⁻, including regulation of membrane transport, their ability to synthesize compatible solutes and to deal with reactive oxygen species, are highlighted. Interacting stress factors in addition to salinity, such as heavy metals and flooding, are also topics gaining increased attention in the search to understand the biology of halophytes.

CONCLUSIONS
Halophytes will play increasingly important roles as models for understanding plant salt tolerance, as genetic resources contributing towards the goal of improvement of salt tolerance in some crops, for re-vegetation of saline lands, and as ‘niche crops’ in their own right for landscapes with saline soils.

Key words: Salinity, salt tolerance, halophyte, extremophile, stress tolerance, salt marsh, NaCl, ion transport, ion compartmentation, plant water use, osmotic adjustment, osmolytes.

INTRODUCTION
Plants have evolved on the Earth to grow in what we, as humans, see as extreme environments; from tundra to tropical forests and from deserts to swamps and even oceans. There are species that tolerate cold, heat, drought and floods. In all cases liquid water is essential, for growth not only as the medium of metabolism, but also as the medium of transport within the plant. Without the bulk flow of liquid water, minerals cannot be transported from roots to shoots and fixed carbon from shoots to roots. Some plants survive freezing and dehydration, but they do not grow without liquid water. Water is plentiful on the planet: there is a staggering 280 kg of water cm⁻² of the surface (Goldschmidt, 1954) but this is mostly in the oceans and large areas of land have little fresh water. Nevertheless, most of the land surface, with its variety of soil types, is well populated with plants belonging to any one of about 351,000 species (species whose names are agreed by taxonomists: http://www.theplantlist.org/). The vast majority of these plants, however, cannot grow in the most plentiful supply of water on the planet, seawater.

Most (72%) of the surface of the Earth is covered in a salt solution dominated by Na⁺ and Cl⁻. Ocean waters commonly contain about 19 g of Cl⁻ and 11 g Na⁺ kg⁻¹ of solution (about 560 mm Cl⁻ and 480 mm Na⁺); there are also substantial concentrations of Mg²⁺ (55 mm), SO₄²⁻ (29 mm), K⁺ (10 mm) and Ca²⁺ (10 mm) but, amongst the essential mineral nutrients for plants, very low concentrations of H₂PO₄⁻/HPO₄²⁻ and NO₃⁻ (Harvey, 1966). It is the substantial concentrations of Cl⁻ and Na⁺ in seawater that are inimical to the growth of most, but not all, plants. The exceptional plants are called halophytes. Vascular halophytes inhabit near-shore shallows and estuaries (e.g. seagrasses), land within the tidal zone (e.g. mangrove forests), coastal salt marshes, inland salt lakes and saline deserts.

Amongst plants there is a continuum of tolerance to Cl⁻ and Na⁺ in their environment, from the very sensitive (e.g. chickpea; Flowers et al., 2010b) to the tolerant (e.g. some Tecticornia species; English and Colmer, 2013). While there has been debate about just where a limit is drawn defining halophytes (see Flowers and Colmer, 2008), at the upper end of tolerance for land plants are the eulahophytes, plants that can tolerate repeated exposure to seawater in the root-zone (Breckle, 2002; Flowers and Colmer, 2008). Such plants are quite rare: there are probably less than 500 species that can grow in salt concentrations of the ‘average’ seawater (Flowers et al., 2010a). The low frequency of occurrence of halophytes (about 0.14% of named plant species) is at first sight surprising since at the time at which plants colonized the land, seawater contained substantial salt concentrations (about 30 g kg⁻¹; Railsback et al., 1999). Colonization of the land required adaptation to the very low free energy of water in the atmosphere in a process that probably occurred at the margins of freshwater pools some 470 million years ago (see Flowers et al., 2010a). The rather small number of halophytic species suggests salt tolerance is not a fundamental trait in terrestrial plants and that salt tolerance appeared later during their evolution.
Halophytic plants are, then, the flora of saline environments. The physiology of halophytes, with its focus on adaptations enabling these fascinating plants to live in challenging environments that the vast majority of species cannot inhabit, is discussed by the authors of the papers in this Special Issue on ‘Halophytes and Saline Adaptations’. The evolution of halophytes, the mechanisms by which they deal with water and ion transport and how they cope with a combination of stress factors such as heavy metals and flooding in addition to salinity, are all considered. Although the focus is on plant adaptations, papers in this Special Issue also show that further improvements in knowledge of halophytes and their mechanisms might be applied to develop more salt-tolerant crops – either conventional crops or halophytic species – and to enhance re-vegetation of degraded lands.

RECENT ADVANCES IN UNDERSTANDING HALOPHYTE EVOLUTION AND SYSTEMATICS

In her paper, Bromham (2015) notes the rarity of halophytes, but that they are widely distributed across the families of flowering plants as they have evolved independently in many lineages. Salt tolerance developed early in some lineages and in others there have been many origins. Salt tolerance, however, occurs in relatively few current species, suggesting this trait has either been gained and lost, or that various salt-tolerant species become extinct over evolutionary time. In answering the question, ‘what phylogenetic analysis of halophytes can tell us about the macroevolution of salt tolerance’, Bromham concludes that salt tolerance may be more likely where enabling traits, such as C₄ photosynthetic metabolism, have already evolved. Saslis-Lagoudakis et al. (2015) examine whether such ‘enablers’ might aid the evolution of tolerance to more than one stress – that ‘traits related to tolerance to one type of stress can facilitate the evolution of another type of stress resistance’. They use data from the National Geochemical Survey of Australia to predict soil pH and electrical conductivity at sites where grasses were recorded in the Atlas of Living Australia (http://www.ala.org.au) and test for a correlation between predicted salinity and alkalinity. Despite limitations in the available data, they find ‘grass taxa in areas of high predicted-salinity also tend to be found in conditions of high predicted-alkalinity’, a finding consistent with the presence of enabling traits promoting evolution of tolerance to stress. Steffen et al. (2015) evaluate the phylogeny of two of the most salt-tolerant genera of flowering plants, Salicornia and Sarcocornia, genera that probably appeared in Eurasia in the Middle Miocene period, some 16–12 million years ago. These two genera have long presented difficulties for taxonomists because of their growth form with highly reduced leaves and flowers. Based on analysis of DNA from herbarium specimens they show that the Salicornia/Sarcocornia lineage diversified into four subclades, one of which includes Salicornia and the other three American, Eurasian and South African/Australian Sarcocornia. Sarcocornia has repeatedly evolved similar prostrate forms and is tolerant of flooding, as well as having high salinity tolerance. Cortinhas et al. (2015) look at another taxonomically difficult genus, Limonium. Morphometric analysis of plants collected from Portugal and Spain show L. narbonense to occur on the coasts of Portugal, as well as identifying a new species, L. maritimum. Plants of both species are found in mixed populations, where hybridization, introgression and apomixis are facilitated. They interpret their data as suggesting the species are relatively new and still evolving, perhaps something that is common among halophytes.

MANAGING SALT AND WATER RELATIONS: HALOPHYTE PHYSIOLOGY AND BIOCHEMISTRY

Plants transpire approximately 750 g water to fix 1 g C, and since C is about 45 % of the dry matter, gaining 1 g of dry weight involves the loss of some 1-7 kg water (calculated from Taiz and Zeiger, 2006). If this water were seawater, then 58 g salt would be accumulated per g dry weight gain, equivalent to about 1 mol of NaCl for every 9 g water (water content 90 % of fresh weight), and result in the precipitation of NaCl in the leaves! Patently, this does not happen and halophytes are able to reduce the concentration of ions present in solution in the xylem so that the quantity of salt delivered to the leaves can be accommodated by growth (for some halophytes ion excretion via salt glands is also a factor). All plants are able, up to a point, to regulate the ions entering the xylem stream and this ability may be a determining factor in salt tolerance (Muens and Tester, 2008): halophytes are able to achieve this Na⁺ and Cl⁻ ‘exclusion’ at high external salt concentrations (Flowers and Colmer, 2008). Removal of Na⁺ from the xylem sap (also called ion retrieval) as it ascends to the leaves can also contribute to genotypic differences in ‘apparent exclusion’ of Na⁺ from entering leaves (Muens and Tester, 2008). Reef and Lovelock (2015) tabulate the degree of ‘exclusion’, which ranges from 90 to 99-6 % of the external Na⁺, among mangroves – tropical and subtropical trees growing in tidal wetlands. In mangroves, Na⁺ ‘exclusion’ from the xylem is achieved through anatomical adaptations that reduce or prevent apoplastic movement of solution from outside the roots to the xylem, ensuring that cellular membranes and their transporters can then determine the ions that pass into the xylem. About one-third of the species for which they tabulate data also have salt glands that secrete ions from the leaves.

There is still much to be learned about the regulation of water uptake, especially when the external root-zone conditions encompass not only salinity but also anoxia, for which specific root adaptations enhancing internal aeration are also of key significance. Nguyen et al. (2015) investigate the effects of a salinity gradient on the growth of the grey mangrove, Avicennia marina. Plants are grown hydroponically and measurements of both leaf gas exchange and the ratio of 13C to 12C in the biomass are used to evaluate the effects of salinity on water use. Growth is optimal in 50–75 % seawater and poor in the absence of salt. Gas exchange follows a similar pattern, but the instantaneous water use efficiency is similar across the salinity gradient. Anatomical studies reveal effects of salinity on the development of xylem vessels, with estimated maximal hydraulic conductance in seedlings grown in 75 % seawater and minimal conductance in those grown without salt. Salinity has more than an instantaneous effect on the physiology, influencing the differentiation of the xylem vessels, and confirming the
importance of long-term studies on the response of halophytes to salinity.

Many halophytes grow in areas that are not only saline but, like the mangroves discussed above, are also prone to standing water. Konnerup et al. (2015) investigate submergence tolerance in two species of the highly salt-tolerant ‘stem succulent’ (i.e. reduced and fused leaves, as in Sarcocornia mentioned above) Tecticornia, growing in ephemeral salt lakes. Tecticornia medusa is more tolerant of submergence than T. auriculata, a finding consistent with the occurrence of the much shorter-statured T. medusa in the more flood-prone inner areas of the marsh. The greater submergence tolerance of T. medusa is in spite of it having lower O2 concentrations within the succulent tissues than in those of T. auriculata, when submerged in the dark. In the light, underwater photosynthesis increases the O2 concentrations within the succulent tissues of both species. The succulent shoots of both species contain high concentrations of Na+ and Cl− so that submergence in water of low salinity results in a strong gradient for water entry. In the case of T. auriculata this results in substantial tissue swelling and even the rupture of cells, whereas T. medusa resists swelling and hence is not damaged. The authors speculate that a less permeable cuticle might be one factor that reduces swelling of the succulent tissues and could also contribute to the lower internal O2 status of the succulent stems of T. medusa. The stems of T. medusa cope with the interesting combination of both high cellular Na+ and Cl− concentrations as well as short-term severe hypoxia during the dark period when submerged.

While many halophytes have to deal with flooding, all have to adjust to the salinity of their external environment. Flowers et al. (2015) summarise the solute compartmentation model of salt tolerance, in which it is hypothesized that osmotic adjustment occurs primarily with the ions present in the environment (mostly Na+ and Cl−), but due to their potential disruption of metabolism if present at high concentrations (‘toxicity’), these ions are concentrated in the vacuoles, relative to the cytosol (compatible organic solutes accumulate in the cytoplasm). They evaluate data on the concentrations of Na+ and Cl− in chloroplasts and cytosol (there are no data for plant mitochondria) and conclude that chloroplastic Na+ concentrations are regulated at between 100 and 250 mM, similar to the concentrations found to be present in the cytosol for the few cases in which this has been evaluated. The authors discuss the concept of ‘tissue tolerance’ of high Na+ (and Cl−) in relation to halophytes. However, whether the concentrations of Na+ and Cl− are ever toxic when halophytes are exposed to supra-optimal salt concentrations is not clear; other factors such as low cytoplasmic K+ concentrations, high Mg2+ concentrations, and decreased stomatal conductance limiting net photosynthesis, hormonal imbalances, increased damage from reactive oxygen species, and the energetic requirements of either ion transport or the synthesis of compatible solutes (or both) could contribute to reduced growth and eventual death. Slama et al. (2015) address the role of organic solutes (also termed ‘osmolytes’) in the cellular compartmentation model, reviewing the diversity of these compatible solutes (amino acids, quaternary ammonium compounds, taurine, sulphonium compounds, sugars and sugar alcohols) found in halophytes. They describe the distribution amongst the main families in which halophytes are found (Amaranthaceae, Plumbaginaceae, Plantaginaceae, Aizoaceae, Poaceae, Brassicaceae), the pathways of synthesis and the roles in osmotic adjustment. Osmolytes function as chemical chaperones and as scavengers of hydroxyl radicals. Also considered are the regulation of synthesis of osmolytes, their transport and whether salt tolerance might be enhanced through the use of transgenics or by exogenous application to increase these compounds in plant tissues. The potential economic value of halophytes as sources of pharmaceuticals and medicines is also discussed.

In modulating water transport to reduce the net uptake of salts to the shoots, halophytes operate with reduced stomatal opening and with consequences for generation of reactive oxygen species (ROS). Uzilday et al. (2015) report the results of their investigation into the ability of halophytes to deal with ROS. Taking advantage of the advances in plant science made using the model species Arabidopsis thaliana, they use a close relative Eutrema parvulum, which is salt tolerant. They expose plants to salt and measure the response of growth, tissue water and ion concentrations, as well as proline levels and the expression of genes involved in its metabolism, the activities of antioxidant enzymes of the water–water cycle (superoxide dismutase, peroxidases and ascorbate peroxidases, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase) as well as H2O2 and lipid peroxidation in whole leaves and in chloroplasts, the main site of production of ROS. Eutrema parvulum tolerates NaCl (300 mM), with tissues showing elevated Na+ and Cl− concentrations and reduced concentrations of Ca2+ and K+, whereas proline levels increase. They conclude that the water–water cycle enzymes might prevent accumulation of excess ROS in chloroplasts and therefore protect the photosynthetic machinery. One of the consequences of the generation of ROS is the accumulation of highly toxic alkaldehydes, which are metabolized by aldehyde dehydrogenases (ALDH). Hou and Bartels (2015) investigate aldehyde scavengers using protein sequences of all known ALDH families in a genome-wide search in Eutrema parvulum and E. salsugineum. They find 16 ALDH genes from E. parvulum and 17 from E. salsugineum, which are assigned to ten of the 24 known families of ALDH. Phylogenetic analysis of ALDH protein sequences indicates that Eutrema ALDHs are closely related to those of Arabidopsis, most having similar expression patterns in saline conditions. The difference between two ALDHs between A. thaliana and the two Eutrema species suggests that the halophytes have modified regulatory pathways that may contribute to their tolerance of salt.

Key to salt tolerance is the ability of halophytes to regulate the uptake of Na+ and Cl− while maintaining cytoplasmic K+ and Mg2+ concentrations at levels required for activation of essential enzyme activities. Bose et al. (2015) use two halophytes, Atriplex lentiformis and Chenopodium quinoa, to investigate responses of root cell membrane potential and net H+/Na+ and K+ fluxes to NaCl-salinity. Exposure to 100 mM NaCl results only in small depolarizations of the root cell membrane potential owing to a fast response of the plasma membrane H+ ATPase to restore the membrane potential and H+ electrochemical gradient that drives secondary-active ion transport such as Na+/H+ antiport, which contributes to regulation of Na+ net uptake. The importance of K+ retention, rather than net K+ loss, for cytosolic ion homeostasis is emphasized. Responses of these two
halophytes are compared with those of the non-halophyte Arabidopsis thaliana, which suffers greater depolarizations and net K⁺ loss when subjected to NaCl.

As we have already noted, a key aspect of salt tolerance is the ability to compartmentalize ions within vacuoles, a process that involves ion transporters at the tonoplast (vacuolar membrane) and particularly vacuolar Na⁺/H⁺ exchangers (NHX). Yuan et al. (2015) investigate the vacuolar NHX in a highly salt-tolerant species, Zygophyllum xanthoxylum, collected from the arid regions of China. They use post-transcriptional gene silencing of ZxNHX to investigate its role in monovalent cation homeostasis. Plants are treated with NaCl (50 mM) and effects on growth, osmotic adjustment, photosynthesis and water use efficiency are evaluated. Comparison of expression levels of enzymes involved in ion transport (ZxSOS1, ZxHKT1;1, ZxAKT1 and ZxSKOR) between wild-type and ZxNHX-silenced lines show ZxNHX to play a role at various levels in Na⁺ and K⁺ transport and homeostasis, including an integrated regulation of the altered activity of ZxNHX on expression and activity of genes of other proteins involved in Na⁺ and K⁺ transport.

‘PUTTING HALOPHYTES TO WORK’

While halophytes are intrinsically fascinating for the ways in which they are adapted to grow under conditions lethal for most plant species, this ability has increasing potential in a world where the human population is still increasing and land-use practices and changes in the climate are likely to lead to increased salinization of the land surface in many regions (Rozema and Flowers, 2008). Salinization often occurs alongside the accumulation of other pollutants and halophytes have been used in various locations around the world in projects to re-vegetate saline soils, with environmental benefits. Lutts and Lefèvre (2015) review literature that demonstrates that some halophytes not only cope with high salinity in substrates being re-vegetated, but can also tolerate heavy metals. Heavy metals can occur at elevated levels, for example, in some salt marshes and in some mine tailings. Their review provides a comprehensive list of various species and metal combinations for which data currently exist, and highlights a potential role for various halophytes to sequester metal ions in some soil remediation situations. The influence of high NaCl on metal chemistry in soils and plant absorption are discussed, and so are plant tolerance mechanisms as studied to date in some halophytes. Regulation of metal translocation and metal chelation within plants, and biochemical responses to metal ions including oxidative stress responses, are reviewed. The findings are considered in the context of the use of halophytes in land remediation for sites with heavy metal pollution.

Ventura et al. (2015) review the history of growing crops with salt waters, both conventional crops and halophytes. Recent development began in the 1960s with the work of Hugo and Elisabeth Boyko: since then, there has been sporadic interest in developing halophytes as crops. Ventura and colleagues document the creation of the HALOPH database (which is now available as eHALOPH at http://www.sussex.ac.uk/affiliates/halophytes/) and discuss their potential uses for forage/fodder, food and gourmet vegetables, in landscaping and as ornamental plants, and for industrial uses as well as a means to treat saline effluents. In spite of the small number of species, halophytes are a considerable resource, given the almost limitless supplies of salt water available on earth. Song and Wang (2015) review the potential of one halophyte, Suaeda salsa, as source of food, medicine and forage. Suaeda salsa, like some other halophytes, produces two types of seed – hard black seeds that are sensitive to salinity and soft brown seeds with green cotyledons that can germinate in NaCl (600 mM) – and so provides a useful model for understanding the effects of salinity on germination. Suaeda salsa is also a source of genes that may play important roles in salt tolerance: 22 have been cloned, many of which are involved in ion homeostasis. Song and Wang conclude by describing the potential for utilization of S. salsa as a food, a medicine and in the restoration of contaminated and salinized land.

CONCLUSIONS

Halophytes are remarkable plants whose potential is yet to be exploited. Their use as food for humans and forage/fodder for livestock may be compromised by the high salt concentration of the vegetative tissues, although systems can be developed using feed mixes for livestock ruminants (Norman et al., 2013). A high salt concentration in the shoots of halophytes is not, however, a constraint for the use of their seed, which are lower in salt, particularly as sources of oils (e.g. Glenn et al., 2013). The presence of salt in the vegetative tissue should not be a constraint to the use of halophytes as a source of compounds for industrial or pharmaceutical use. Halophytes may also be useful as a means to enhance the tolerance of conventional crops, both in terms of understanding target traits and/or as a genetic resource (e.g. Colmer et al., 2006). Although there has been little success so far in the use of genes from halophytes to enhance salt tolerance of grain crops, this may change as an ability to transfer, not one, but a suite of genes from one species to another is developed. Some 20 years ago, Flowers and Yeo (1995) questioned whether breeding for salt tolerance was a worthwhile exercise and asked, ‘Another important question is whether, at least in some cases, effort would not be better spent on domestication of halophytes rather than on improving resistance of conventional crops’. With the human population of the world continuing to grow (estimated to reach 9 billion in the next 25 years and 9.6 billion in 2050; http://faostat3.fao.org/home/E), the question is even more relevant today. We are likely to need all our resources if we are to maintain food supply over the coming years: halophytes will play an increasingly important role both as models for generating tolerance and as ‘niche crops’ in their own right for landscapes with saline soils.

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LITERATURE CITED


