

Putting halophytes to work – genetics, biochemistry and physiology

Bernhard Huchzermeyer^A and Tim Flowers^{B,C,D}

^AInstitute of Botany, Leibniz Universitaet Hannover, Herrenhaeuser Str. 2, 30419 Hannover, Germany.

^BJohn Maynard Smith Building, School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, UK.

^CSchool of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

^DCorresponding author. Email: t.j.flowers@sussex.ac.uk

Abstract. Halophytes are a small group of plants able to tolerate saline soils whose salt concentrations can reach those found in ocean waters and beyond. Since most plants, including many of our crops, are unable to survive salt concentrations one sixth those in seawater (about 80 mM NaCl), the tolerance of halophytes to salt has academic and economic importance. In 2009 the COST Action *Putting halophytes to work – from genes to ecosystems* was established and it was from contributions to a conference held at the Leibniz University, Hannover, Germany, in 2012 that this Special Issue has been produced. The 17 contributions cover the fundamentals of salt tolerance and aspects of the biochemistry and physiology of tolerance in the context of advancing the development of salt-tolerant crops.

Additional keywords: heavy metals, microbiome, proteomics, reactive oxygen species, ROS, salinity, salt tolerance, water logging.

It has long been known that some plants are salt tolerant (see for example Waisel 1972), but it was not the early twentieth century that halophytes were defined – as plants that are exposed at least during a period of their life to salt concentrations that the majority of plants will not survive (Stocker 1928). Since then there have been various attempts to refine what we understand as halophytes. For example, Braun-Blanquet (1931), categorised halophytes as oligo-, meso- and euhalophytes dependent upon their salinity tolerance: obligate (eu-) halophytes depend on salinity, semi obligate (meso-) halophytes show improved growth rate in presence of salt and oligo-halophytes show improved performance in the absence of salt but survive salt concentrations toxic for most plants. Chapman (1942) characterised the coastal vegetation of the United Kingdom by the salt concentration of the soil water phase, defining halophytes as plants that are able to complete their life cycle in the presence of salt concentrations exceeding 0.5% NaCl (~86 mM). He suggested the term euhalophytes be restricted to those plants whose optimal growth takes place in an environment in which there is more than 0.5% NaCl; miohalophytes he defined as those plants that are to be found, either commonly or rarely, in habitats where there is more than 0.5% NaCl. Since Chapman's (1942) review a variety of definitions and schemes of classification have been published (see, Breckle 2002; for further discussion) with Flowers and Colmer (2008) increasing the (arbitrary) dividing line between halophytes and glycophytes to 200 mM NaCl. While the concept of salt tolerance in plants is clear, definitions remain problematic because of the variety of environments in which salt-tolerant plants grow.

Progress in understanding how halophytes can grow in salt water has also been slow to develop, but by the early part of the

twentieth century it was clear that osmotic adjustment was important to salt tolerance. Schimper (1898) had argued that salt prevents plants from taking up water and predicted that salinity should lead to a reduced transpiration rate. However, by the early part of the twentieth century, there was controversy as Stocker (1925), in experiments with *Aster tripolium* and *Salicornia herbacea*, and Montfort (1926) and Montfort and Branderup (1927a, 1927b), analysing salt marshes on the coastline of the Baltic Sea, could not find the reduced transpiration rate predicted by Schimper. Montfort and Branderup (1927b) also pointed out that cellular physiology needed to receive more attention. Subsequently, various ecological studies confirmed high concentrations of salts in the cells of halophytes (e.g. Berger-Landfeld 1933; Montfort 1937; Pampe 1940; Steiner 1934, 1939). Thus during the mid-twentieth century it became clear that the osmotic pressure within cells was high enough to allow water uptake even in a saline environment.

It was as part of the analyses of osmotic pressures that organic components of cell sap were first measured. Early studies were restricted to sugars and organic acids (see, for example, Steiner 1939; Steiner and Eschrich 1958), but as analytical techniques improved a range of biochemical components were implicated in salt tolerance (e.g. Storey and Wyn Jones 1975; see, Flowers Troke *et al.* 1977). Since the early 1980s techniques have continued to develop, along with greater knowledge of the cellular basis of salt tolerance (e.g. Greenway and Munns 1980; Flowers Hajibagheri *et al.* 1986). We now know there are just a few thousand of the quarter of a million plant species that can survive in about one sixth the concentration of sodium and chloride found in seawater (Aronson 1989; Menzel and

Lieth 2003) and only a few hundred (Flowers Galal *et al.* 2010) that can survive seawater salt concentrations (around 500 mM NaCl).

Since most of our crops are quite salt sensitive, agriculture has to compete with domestic and industrial use for fresh water, which is likely to become an increasingly valuable commodity in the future. Changes in the global climate are predicted to increase variability in weather patterns and enhance drought in some regions, with an attendant need for providing water for plant growth. Consequently, any savings of fresh water that can be made by utilising salt water, which is available in abundance, could make a valuable contribution to future agriculture. It was with the aim of improving our understanding of salt tolerance in plants that a COST Action on ‘*Putting halophytes to work – from genes to ecosystems*’ was established in 2009 as a basis for enabling the use of saline water in agriculture. Part of that process was to investigate the ‘*Proteomics, genetics, bioinformatics and metabolomics of halophytes*’. The papers in this Special Issue of *Functional Plant Biology* were presented at a conference funded by COST and held at the Leibniz University, Hannover, Germany, in 2012; they address major aspects of the biochemistry of salt-tolerant plants.

Cheeseman (2013) addresses the complexity of the adaptations to salinity in halophytes in terms of seven plant functions that have to be integrated in a halophyte (Cheeseman 2013; fig. 1). His conclusion is that new ‘omic’ approaches should provide a greater understanding of the complexities of the response, providing the data are not gathered from experiments involving sudden, often lethal, shock: a critical component of resolving these adaptations will be improving our understanding of the nature of the cytosol and the activities of ions within it. Two following papers address changes in the proteomes of halophytes in response to salt. Kosová *et al.* (2013) compare differences observed in salt response of closely related species that differ in salt resistance. The two most important results of this study are: (i) salt-resistant plants display an enhanced constitutive expression of several salt-responsive genes, and (ii) they show few salinity-related disturbances in energy metabolism. Koyro *et al.* (2013) compared proteomic data of experiments applying different salt concentrations to halophytes. Results indicate that increased cellular metabolite concentrations (proline, for instance) are generally not directly related to the abundance of enzymes of the last steps of their catalytic pathways. In cases where plants showed optimal growth rates at moderate salt concentrations, proteins could be identified showing corresponding patterns of optimal abundance at the salt concentration optimal for plant performance. An important conclusion was that keeping structural integrity of the cytosol is of high importance for optimal plant growth.

The biochemistry of halophytes and differences from glycophytes is considered in four papers. Gil *et al.* (2013) ask ‘Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes?’ and highlight problems in unequivocally attributing a biological role to soluble carbohydrates in the salt tolerance mechanisms of particular species. Soluble carbohydrates not only have roles in the osmotic balance, but are key metabolites and signalling molecules with a “wide variability in the responses to salt stress observed in different species, without any clear,

quantitative or qualitative general patterns of accumulation of specific sugars or polyalcohols”. They question whether the results of laboratory experiments can be extrapolated to the field and advocate more effort in analysis of plant collected from their natural habitats. Bartels and Dinakar (2013) focus on comparing physiological traits of halophytes and glycophytes. They tabulate differences between *Arabidopsis* and *Thellungiella* related to photosynthesis, sodium fluxes, metabolic and transcript changes, and antioxidant enzymes. Their arguments are based on the observation that since halophytes have evolved in many taxons differing in metabolism and physiology, it may be expected that these plants differ in their strategies to adapt to high salt concentrations. Identification of these strategies may lead to a better understanding of the phenomenon of salinity tolerance, and allow more efficient approaches to improve salinity tolerance of crops. Ozgur *et al.* (2013) develop the importance of antioxidant systems in discussing reactive oxygen species: regulation and antioxidant defence in halophytes. They describe recent advances in the acclimation of halophytes to reactive oxygen species and discuss their role in signalling and in the response of halophytes to salt, again highlighting the role of ‘omic’ approaches to the study of the complex relationships among antioxidants and their functions in halophytes. Kranner and Seal (2013) include in their analysis of salt stress, signalling and redox control in seeds, salt effects on the mother plant and implications for seed development. They introduce a three-phase (alarm, resistance and exhaustion) model to explain stress in seeds and discuss the implications of redox control for future research on salt-stress biology of seeds. Many of the proposed approaches may be applied to halophyte research in general.

The importance of ion homeostasis is developed in two contributions. Ahmed *et al.* (2013) report optimal growth of the halophytic grass *Aeluropus lagopoides* to occur at relatively low (26 mM) salt concentration: increasing salt decreased growth, and photosynthesis. A decrease in transpiration helped to minimise Na⁺ uptake and this, together with increased secretion from salt glands and the upregulation of membrane transport proteins (V-NHX and PM-NHX), enabled *A. lagopoides* to compartmentalise Na⁺ at salinities up to 373 mM NaCl and maintain K⁺ homeostasis to this external salt concentration. The importance of K⁺ homeostasis in the response of plants to salinity and waterlogging is reviewed by Barrett-Lennard and Shabala (2013). Waterlogging generally exacerbates the effects of salinity on growth through its effects on ion uptake. Comparisons across glycophytes and halophytes show that hypoxia, with its low partial pressures of O₂, increases Na⁺ and Cl[−] concentrations and decreases K⁺ concentrations in shoots of plants growing in saline conditions. Using data in the literature from 22 species, they conclude that decreases in growth are not only due to increases in Na⁺ and Cl[−], but also to decreases in K⁺ concentrations. The reasons for the decrease in K⁺ (reduced uptake or increased efflux) need further investigation, especially since, in contrast to mammalian systems, almost nothing is known about the effects of the partial pressure of oxygen on plant ion channels.

Water-logging is just one of the other stresses that can affect halophytes and Hamed *et al.* (2013) have reviewed literature on halophyte responses to simultaneously occurring stresses. They give special attention to the interaction of salinity with drought,

heavy metal and nutrient deficiencies. A second topic of their review was to analyse pretreatment of halophytes with one stressor and subsequently observe responses to a second stress event. They found that the response to multiple stresses does not reflect an additive effect. Moreover, pretreatment at an early stage of development with one type of stress improves stress tolerance to salt at a later period of plant growth. English and Colmer (2013) report the extreme tolerance of two halophytes found around salt lakes in Australia. *T. pergranulata* subsp. *pergranulata* occupies more saline sites than *T. indica* subsp. *bidens*, but both species can tolerate 2 M NaCl in glasshouse experiments where the plants were grown in well drained sand. Both species were able to maintain adequate K⁺ concentrations, and the use of C₄ photosynthesis by *T. indica* may be part of an explanation for its location in drier areas than *T. pergranulata*, which uses the C₃ pathway.

Continuing the theme of tolerance to multiple stresses, Redondo-Gómez (2013) reviewed the literature on the accumulation of heavy metals in the genus *Spartina*, most species of which are salt tolerant. She compared data on 10 of 18 species and found that on average heavy metal concentrations were lower in above-ground than below-ground tissues and in the surrounding medium. However, below-ground tissues of *S. maritima* and *S. alterniflora* had higher Cd, Cu and Zn concentrations than those in the soil. Some species (e.g. *S. argentinensis*, *S. densiflora*, *S. densiflora*, *S. maritima*) accumulate significant concentrations of heavy metals and are possible candidates for use in phytoremediation. Duarte *et al.* (2013) analysed heavy metal tolerance of halophytes in two salt marshes in Portugal with a view to using halophytes as bio-indicators of heavy metal contamination of estuaries. They concluded that *S. maritima* could be used as a potential bio-indicator of heavy metal contamination. Couto *et al.* (2013) analysed the heavy metal contents of *Scirpus maritimus*, *S. maritima* and *Zostera noltii* growing in a relatively unpolluted estuary and showed that because of the large areas occupied by these species they contributed significant quantities of heavy metals to the surrounding water body.

Ruppel *et al.* (2013) review the adaptations to salinity seen in the Fungi, Bacteria and Archaea and note that while the microbiome of glycophytes has been well researched over the last 10 years, very little is known of the microbiome of halophytes. From the few studies available, specialised microorganisms appear to be associated with halophytes. Although the amount of data is small, their review suggests how microbes could influence response of halophytes to salinity, an area ripe for further research especially given the potential economic significance of halophytes (Rozema *et al.* 2013). Buhmann and Papenbrock (2013) develop the ideas of using the secondary compounds present in halophytes as commercial products. They present an overview on strategies to use such compounds from halophytes, many of which are directly involved in stress tolerance by acting as antioxidants or compatible solutes. They observed that the abundance of such metabolites varies with growth conditions, so that defining the cultivation conditions optimal for production of these metabolites is important. Ventura *et al.* (2013) show that repeated harvesting of halophytes grown as a cash crop results in the development of chlorosis, which could be reduced by the correct formulation of

added Fe as a nutrient. Their data highlight the importance of growth conditions – salinity and Fe supply – in optimising the production of any halophyte as a cash crop.

Halophytes represent a small and specialised group of plants able to tolerate not only the salinity that reduces crop yields in many of our agricultural systems (Munns and Tester 2008), but also waterlogging (Colmer and Flowers 2008) and other environmental conditions (as evidenced in this Special Issue). As such halophytes offer a means to understand how plants can tolerate salt; an understanding that can be used in enhancing the salt tolerance of crops, a trait that is likely to be invaluable in a future hotter drier world with greater variations in weather than we currently experience. However, halophytes not only offer insights into the mechanisms of salt tolerance but practical means to address reclamation of salinised soils as well as their direct use in saline agriculture (Rozema and Flowers 2008; Rozema *et al.* 2013). As such they are plants with considerable potential and worthy of further investment.

Acknowledgements

BH and TJF, as Editors of the Special Issue, would like to acknowledge the support of COST Action FA0901, ‘Putting halophytes to work – genetics, biochemistry and physiology’. The Action enabled participation in a conference held at the Leibniz University, Hannover, Germany, on 29 and 30 August 2012 to which the contributions to this Special Issue were presented.

References

- Ahmed MZ, Shimazaki T, Gulzar S, Kikuchi A, Gul B, Khan MA, Koyro H-W, Huchzermeyer B, Watanabe KN (2013) The influence of genes regulating transmembrane transport of Na⁺ on the salt resistance of *Aeluropus lagopoides*. *Functional Plant Biology* **40**, 860–871. doi:10.1071/FP12346
- Aronson JA (1989) ‘HALOPH A Data Base of Salt Tolerant Plants of the World.’ (Office of Arid Land Studies, University of Arizona: Tucson, Arizona) 77.
- Barrett-Lennard EG, Shabala SN (2013) The waterlogging/salinity interaction in higher plants revisited – focusing on the hypoxia-induced disturbance to K⁺ homeostasis. *Functional Plant Biology* **40**, 872–882. doi:10.1071/FP12235
- Bartels D, Dinakar C (2013) Balancing salinity stress responses in halophytes and non-halophytes: a comparison between *Thellungiella* and *Arabidopsis thaliana*. *Functional Plant Biology* **40**, 819–831. doi:10.1071/FP12299
- Berger-Landfeld U (1933) Zur Frage der “Physiologischen Trockenheit” der Salzböden. *Beiheft zum Botanischen Centralblatt* **51**, 697–710.
- Braun-Blanquet J (1931) Zur Frage der “Physiologischen Trockenheit” der Salzböden. *Berichte der Schweizerischen Botanischen Gesellschaft* **40**, 33–39.
- Breckle SW (2002) Salinity, halophytes and salt affected natural ecosystems. In ‘Salinity: Environment-Plants-Molecules’. (Eds A Lächli A, U Lüttge) (Kluwer: Dordrecht).
- Buhmann A, Papenbrock J (2013) An economic point of view of secondary compounds in halophytes. *Functional Plant Biology* **40**, 952–967. doi:10.1071/FP12342
- Chapman VJ (1942) New perspectives in the halophytes. *The Quarterly Review of Biology* **17**, 291–311. doi:10.1086/394660
- Cheeseman JM (2013) The integration of activity in saline environments: problems and perspectives. *Functional Plant Biology* **40**, 759–774. doi:10.1071/FP12285
- Colmer TD, Flowers TJ (2008) Flooding tolerance in halophytes. *New Phytologist* **179**(4), 964–974. doi:10.1111/j.1469-8137.2008.02483.x

- Couto T, Duarte B, Barroso D, Caçador I, Marques JC (2013) Halophytes as sources of metals in estuarine systems with low levels of contamination. *Functional Plant Biology* **40**, 931–939. doi:10.1071/FP12300
- Duarte B, Santos D, Caçador I (2013) Halophyte anti-oxidant feedback seasonality in two salt marshes with different degrees of metal contamination: search for an efficient biomarker. *Functional Plant Biology* **40**, 922–930. doi:10.1071/FP12315
- English JP, Colmer TD (2013) Tolerance of extreme salinity in two stem-succulent halophytes (*Tecticornia* species). *Functional Plant Biology* **40**, 897–912. doi:10.1071/FP12304
- Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. *New Phytologist* **179**(4), 945–963. doi:10.1111/j.1469-8137.2008.02531.x
- Flowers TJ, Troke PF, Yeo AR (1977) The mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology* **28**, 89–121. doi:10.1146/annurev.pp.28.060177.000513
- Flowers TJ, Hajibagheri MA, Clipson NJW (1986) Halophytes. *The Quarterly Review of Biology* **61**, 313–337. doi:10.1086/415032
- Flowers TJ, Galal HK, Bromham L (2010) Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology* **37**(7), 604–612. doi:10.1071/FP09269
- Gil R, Boscaiu M, Lull C, Bautista I, Lidón A, Vicente O (2013) Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes? *Functional Plant Biology* **40**, 805–818. doi:10.1071/FP12359
- Greenway M, Munns R (1980) Mechanisms of salt tolerance in non-halophytes. *Annual Review of Plant Physiology* **31**, 149–190. doi:10.1146/annurev.pp.31.060180.001053
- Hamed KB, Ellouzi H, Talbi OZ, Hessini K, Slama I, Ghnaya T, Bosch SM, Savouré A, Abdely C (2013) Physiological response of halophytes to multiple stresses. *Functional Plant Biology* **40**, 883–896. doi:10.1071/FP13074
- Kosová K, Vítámvás P, Urban MO, Prášil IT (2013) Plant proteome responses to salinity stress – comparison of glycophytes and halophytes. *Functional Plant Biology* **40**, 775–786. doi:10.1071/FP12375
- Koyro H-W, Zörb C, Debez A, Huchzermeyer B (2013) The effect of hyper-osmotic salinity on protein pattern and enzyme activities of halophytes. *Functional Plant Biology* **40**, 787–804. doi:10.1071/FP12387
- Kranner I, Seal CE (2013) Salt stress, signalling and redox control in seeds. *Functional Plant Biology* **40**, 848–859. doi:10.1071/FP13017
- Menzel U, Lieth H (2003) HALOPHYTE Database Vers. 2.0 update. In 'Cash Crop Halophytes. Vol. 38.' (Eds H Lieth and M Mochtchenko) pp. compact disk. (Kluwer: Dordrecht)
- Montfort C (1926) Physiologische und pflanzengeographische Seesalzwirkungen. I. Einfluß ausgleichender Seesalzwirkungen auf Mesophyll- und Schließzellen. Kritik der Iuinschen Hypothese der Salzbeständigkeit. *Jahrbuch der wissenschaftlichen Botanik* **65**, 502–550.
- Montfort C (1937) Die Trockenresistenz der Gezeitenpflanzen und die Frage der Übereinstimmung von Standort und Vegetation. *Berichte der Deutschen Botanischen Gesellschaft* **55**, 85–95.
- Montfort C, Branderup W (1927a) Physiologische und pflanzengeographische Seesalzwirkungen. II. Ökologische Studien über Leistung und erste Entwicklung der Halophyten. *Jahrbuch der wissenschaftlichen Botanik* **66**, 902–946.
- Montfort C, Branderup W (1927b) Physiologische und pflanzengeographische Seesalzwirkungen. III. Die Salzwachstumsreaktion der Wurzel. *Jahrbuch der wissenschaftlichen Botanik* **67**, 105–171.
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59**, 651–681. doi:10.1146/annurev.arplant.59.032607.092911
- Ozgur R, Uzilday B, Sekmen AH, Turkan I (2013) Reactive oxygen species regulation and antioxidant defence in halophytes. *Functional Plant Biology* **40**, 832–847. doi:10.1071/FP12389
- Pampe E (1940) Beiträge zur Ökologie der Hiddenseer Halophyten. *Beiheft zum Botanischen Centralblatt* **60**, 223–326.
- Redondo-Gómez S (2013) Bioaccumulation of heavy metals in *Spartina*. *Functional Plant Biology* **40**, 913–921. doi:10.1071/FP12271
- Rozema J, Flowers T (2008) Crops for a Salinized World. *Science* **322**, 1478–1480. doi:10.1126/science.1168572
- Rozema J, Muscolo A, Flowers TJ (2013) Sustainable cultivation and exploitation of halophyte crops in a salinising world. *Environmental and Experimental Botany* **92**, 1–3. doi:10.1016/j.envexpbot.2013.02.001
- Ruppel S, Franken P, Witzel K (2013) Properties of the halophyte microbiome and their implications for plant salt tolerance. *Functional Plant Biology* **40**, 940–951. doi:10.1071/FP12355
- Schimper A (1898) 'Pflanzengeographie auf physiologischer Grundlagen.' (G Fischer Verlag: Jena)
- Steiner M (1934) Zur Ökologie der Salzmarschen der Nordöstlichen Vereinigten Staaten von Noramerika. *Jahrbuch der wissenschaftlichen Botanik* **81**, 94–202.
- Steiner M (1939) Die Zusammensetzung des Zellsaftes bei höheren Pflanzen in ihrer ökologischen Bedeutung. *Ergebnisse der Biologie* **17**, 152–254.
- Steiner M, Eschrich W (1958) Die osmotische Bedeutung der Mineralstoffe. In 'Handbuch der Pflanzenphysiologie. Vol. 4.' Ed. W Ruhland) pp. 334–354. (Springer: Berlin, Goettingen, Heidelberg)
- Stocker O (1925) Beiträge zum Halophytenproblem. II. Standort und Transpiration der Nordseehalophyten. *Zeitschrift für Botanik* **17**, 1–24.
- Stocker O (1928) Das Halophytenproblem. *Ergebnisse der Biologie* **3**, 265–353. doi:10.1007/978-3-642-91065-4_4
- Storey R, Wyn Jones RG (1975) Betaine and Choline Levels in Plants and their Relationship to NaCl Stress. *Plant Science Letters* **4**, 161–168. doi:10.1016/0304-4211(75)90090-5
- Ventura Y, Myrzabayeva M, Alikulov Z, Cohen S, Shemer Z, Sagi M (2013) The importance of iron supply during repetitive harvesting of *Aster tripolium*. *Functional Plant Biology* **40**, 968–976. doi:10.1071/FP12352
- Waisel Y (1972) 'The biology of halophytes.' (Academic: London)