Review:

Title: Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes?

Running title: Carbohydrates and salt tolerance in halophytes

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Abstract

The induction of biosynthesis and accumulation of osmolytes, including soluble carbohydrates, is a well-known, general response of plants to high soil salinity: they help maintain cellular osmotic balance under salt stress conditions and act as 'osmoprotectants' with chaperon and/or ROS scavenging activities. Yet the ecological relevance and relative contribution of this response to the salt tolerance mechanisms of halophytes in their natural habitats remain largely unknown. In this review, we describe and discuss published data supporting the participation of compatible solutes in those mechanisms, with especial focus on sugars and polyols. We aim to highlight the complexities to unequivocally attribute to carbohydrates a biological role in salt tolerance mechanisms of a given tolerant species. These problems derive from their additional cellular functions (components of primary metabolism, major energy sources and signalling molecules), the difficulties to generalise the results of particular experiments and to compare independently published results, and the scarcity of field studies. As an extension and complement of more common experimental approaches – mostly based on salt treatments of glycophytic models under controlled (but artificial) conditions in laboratory set-ups – we propose to intensify research on halophytes in their natural ecosystems, correlating seasonal changes in soluble carbohydrates contents with the degree of environmental stress affecting the plants, as well as performing comparative analyses in closely related species with different levels of salt resistance. We believe that this strategy will provide novel information that will help to answer the question put forward in the title.

Additional keywords: abiotic stress, environmental stress, metabolomics, saline habitats, stress tolerance.

Introduction: Plant responses to salt stress

High soil salinity is, together with drought, one of the most important environmental stress factors that reduces crop productivity in agriculture and limits plant distribution in nature (Boyer 1982, Hasegawa *et al.* 2000, Bartels and Sunkar 2005, Watson and Byrne 2009). The deleterious effects of salinity for plants are well-known, and are the result of the two components of salt stress: osmotic stress and ionic toxicity. High salt concentrations in the soil solution, by lowering the water potential, cause hyperosmotic shock at the cellular level, with reduced turgor and cell expansion; a sufficiently low water potential in the apoplast can lead to cell dehydration. This effect is not specific for salt stress: other environmental conditions, such as drought, cold, too high temperatures or presence of heavy metals in soil, also cause osmotic stress in plant cells (Munns and Termaat 1986, Zhu 2002, Wahid *et al.* 2007, Thapa *et al.* 2012, Theocharis *et al.* 2012). Salt stress affecting plants is mostly caused by sodium chloride, by far the most abundant salt in the soil solution, and absorbed Na^+ (and also Cl^-) ions are toxic at relatively low concentrations. They inhibit many enzymatic activities and basic cellular processes, such as pre-mRNA processing or protein synthesis, and can directly inactivate proteins and macromolecular structures by interfering with the ionic interactions that maintain their functional conformations (Forment *et al.* 2002, Munns and Tester 2008, Kronzucker and Britto 2011). In addition to these direct, osmotic and toxic effects, excess salt in soil affects plant mineral nutrition by inhibiting the uptake of essential nutrients, such as K^+ and Ca^{2+} (Ashraf 2004, Shabala and Cuin 2007). Finally, and like other stress conditions, high salinity also causes as a secondary effect the generation of 'reactive oxygen species' (ROS); that is, oxidative stress (Halliwell 2006).

Plants have evolved a series of mechanisms that activate in the presence of salt to counteract the above-described harmful effects of NaCl exposure. Intensive research over the last four decades, prompted by the adverse consequences of soil salinity for agriculture – but also by the academic interest of this topic – has allowed to elucidate some of these basic, conserved physiological and biochemical mechanisms of response to salt stress, which are mainly based on: ***i)*** the control of ion transport and ion homeostasis, and the maintenance of cellular osmotic balance, including the compartmentalisation of toxic ions in the vacuole and the synthesis and accumulation of compatible solutes or osmolytes – proline, glycine betaine, sugars or polyols – in the cytosol; ***ii)*** the synthesis of specific 'protective' proteins, such as heat shock proteins, LEA proteins, osmotine, etc.; and ***iii)*** the activation of chemical – e.g., flavonoids and other phenolic compounds, vitamins C and E, carotenoids or GSH – and enzymatic – e.g., superoxide dismutase, catalase, glutathione reductase or several peroxidases – antioxidant systems. Although we specifically refer herein to salt stress, it should be noted that most of these responses are also triggered by all other abiotic stress conditions causing cellular dehydration such as drought, cold or high temperatures (Zhu 2001, Vinocur and Altman 2005, Hussain *et al.* 2008, Flowers and Colmer 2008, Türkan and Demiral 2009, Szabados *et al*. 2011).

There is considerable evidence that all plants, both salt-tolerant and sensitive, utilise the mechanisms outlined above to respond to salt stress (Bartels and Sunkar 2005, Parida and Das 2005, Hussain *et al.* 2008), and it is generally assumed that salt tolerance largely depends on these responses (Glenn *et al.* 1999, Ashraf and Harris 2004, Munns and Tester 2008, Flowers and Colmer 2008). However, there is some confusion in the literature as to the concepts of ***mechanisms of response to salt stress*** and ***mechanisms of salt tolerance***, which are often considered equivalent. The truth is that most wild plants and all crops are glycophytes; that is, salt-sensitive: they are unable to survive when soil salinity exceeds a certain threshold value, which differs for distinct species, but is relatively low. This means that activation of the salt stress response pathways described above does not generally lead to salt tolerance. Somewhat surprisingly, the vast majority of the studies dealing with salt stress responses / salt tolerance mechanisms have been carried out using glycophytes, mostly *Arabidopsis thaliana* – this being the established model in plant molecular biology research – or, to a lesser extent, crops like tobacco, tomato, maize or rice. Physiological, biochemical and molecular responses to saline stress in glycophytes have been extensively investigated and reviewed (e.g., Zhu 2001, Bartels and Sunkar 2005, Munns and Tester 2008, Horie *et al*. 2012). Comparatively much less effort has been invested to study the small percentage of angiosperm species (~ 0.25%, Flowers *et al*. 2010) that are really salt-tolerant – the halophytes – which *a priori* would seem more appropriate models for this kind of studies. Halophytes have been defined as plants specific for natural saline environments, which are able to complete their life cycles in habitats with a level of salinity of at least 200 mM NaCl in soil (Flowers *et al*. 1986, Flowers and Colmer 2008). Nevertheless, many of them can survive salt concentrations equivalent to that of sea water (ca. 500 mM NaCl), or even higher. Of course, this soil salinity threshold is somewhat arbitrary since salt sensitivity continuously varies in plants from typical glycophytic species to extreme halophytes, and there will be always some ‘borderline’ taxa that are difficult to classify according to the above criterion. However, this operational definition is convenient since it excludes most angiosperm species, which will not survive under those conditions. It also seems appropriate to apply the ‘salt tolerance’ concept, *sensu stricto*, only to halophytes, while it would be correct to consider *relative* levels of salt resistance when comparing different glycophytes, or even different cultivars of the same crop species (Grigore *et al.* 2011).

Many halophytes have developed a wide array of anatomical or ecophysiological modifications, often constitutive, but sometimes also salt-induced, as a defence against high soil salinity. They include the presence of salt glands or salt bladders, reduction of leaf area, tissue lignification, increased succulence, or specific photosynthetic adaptations such as the 'kranz anatomy', a cellular structure characteristic of most C_4 plants. Apart from these adaptations, which may contribute significantly to the salt tolerance of particular species, all halophytes respond to salt stress by activating the same pathways used by glycophytes, as mentioned before. For example, plants of the genus *Limonium* have characteristic salt glands which help them get rid of absorbed sodium chloride (Hill and Hill 1973), while other halophytes – e.g., *Atriplex lentiformis* – switch photosynthesis from the C_3 to the C_4 pathway in response to increased external salinity (Zhu and Meinzer 1999). In addition, however, general responses, such as accumulation of compatible osmolytes, have also been described in species of both genera (Briens and Larher 1982, Tipirdamaz *et al.* 2006).

Obviously, there are differences in responses to salinity stress between halophytes and glycophytes, as indicated by the very fact that the former are salt-tolerant, while the latter are not. Yet such differences must be quantitative in nature rather than qualitative. In other words, responses to salt stress are more efficient in halophytes than in glycophytes, although in both cases they may share the same molecular basis (Borsani *et al.* 2003, Pang *et al.* 2010).

Independently of the type of plants investigated, sensitive or tolerant, practically all studies on the mechanisms of defence against high salinity have been performed by applying diverse salt stress treatments to the plant material (in general, NaCl at different concentrations and/or for different times) under controlled conditions in either the laboratory or the greenhouse. The 'response' of plants is then assessed by determining salt-induced changes in different parameters (growth measurements, photosynthetic activity, levels of specific metabolites or proteins, enzymatic activities, expression patterns of specific genes, etc.) as compared to the non-treated controls. Although this experimental approach has provided valuable information on plant responses to salt stress, it is certainly not clear whether an ecological meaning can be ascribed to the laboratory results obtained under artificial conditions, which differ so much from those of plants in their natural habitats (for a more extensive discussion and examples of the limitations of laboratory experiments as compared to fieldwork, see Grigore *et al.* 2011).

In short, we presently have sound knowledge of the different mechanisms used by plants to respond to salt stress but, despite the intensive research on this topic carried out over recent decades, the biological/ecological relevance of these response pathways and their relative contribution to salt tolerance mechanisms in a given tolerant species remain largely unknown. In our opinion, this is partly due to the experimental approaches commonly used, which have led these studies to focus almost exclusively on salt-sensitive species instead of on halophytes, and also on work in laboratory set-ups instead of fieldwork in the natural habitats of plants. We believe that complementary strategies, which analyse the behaviour of halophytes in nature, will help to elucidate the physiological and biochemical mechanisms that are ecologically relevant for salt tolerance.

Many reviews have been published over the last few years, which have dealt with the responses of plants to high soil salinity and/or to other environmental stress conditions and salt tolerance mechanisms, either in general or with emphasis placed on particular aspects (e.g., Flowers and Colmer 2008, Munns and Tester 2008, Türkan and Demiral 2009, Jamil *et al.* 2011, Zhang *et al.* 2012). This review centres on one specific mechanism which, as discussed below, appears to largely contribute to salt tolerance in halophytes: the synthesis and accumulation of compatible solutes under salt stress conditions. As the presence and possible functions of nitrogen-containing osmolytes (proline, glycine betaine) have been generally studied in more detail, and have been the object of recent reviews (Ashraf and Foolad 2007, Chen and Murata 2008, Szabados and Savouré 2010, Grigore *et al.* 2011), we focus specifically on the roles of soluble carbohydrates (sugars and polyols) as osmolytes in salt-tolerant plants. Following the ideas outlined in the previous paragraphs, we aim to comment on published data that support, or not, the possible participation of this type of osmolytes in halophyte responses to salt, and to mainly highlight problems to unequivocally attribute a biological role to soluble carbohydrates in the salt tolerance mechanisms of a particular species, problems deriving from their additional functions as components of primary metabolism and signalling molecules, difficulties to generalise the results of particular experiments and to compare independently published results, and scarcity of field studies.

Functions of osmolytes in salt tolerance mechanisms

Osmolytes in osmotic adjustment: the ion compartmentalisation hypothesis

Salt tolerance seems to be largely dependent on halophytes' capacity to transport the Na^+ and Cl^- ions absorbed by roots to plant's aerial parts. Since these ions are toxic at relatively low concentrations and cannot accumulate in the cytoplasm, it has been proposed that they are sequestered in vacuoles, thus avoiding their deleterious cellular effects; osmotic adjustment under salt stress conditions requires the synthesis and accumulation of osmolytes in the cytoplasm (Flowers *et al.* 1977, Wyn Jones *et al*. 1977, Glenn *et al.* 1999). Osmolytes are very soluble, low-molecular-weight organic compounds, which are considered 'compatible' solutes since they do not interfere with normal metabolism, even at high concentrations. Osmolytes are quite diverse from the chemical point of view, but the most common can be classified into two groups of compounds; firstly, zwitterionic alkylamines, such as amino acids (e.g., proline) and quaternary ammonium compounds (e.g., glycine betaine) (Ashraf and Foolad 2007, Verbruggen and Hermans 2008, Chen and Murata 2011); secondly, polyhydroxylic compounds: soluble carbohydrates such as sugars (sucrose, glucose, fructose, trehalose, etc.), polyols or sugar alcohols (sorbitol, mannitol, pinitol, inositol, etc.) and the raffinose family of oligosaccharides (RFO's, e.g., stachyose and raffinose) (Parida *et al.* 2002, Gavaghan *et al.* 2011). Other less common osmolytes include, for example, tertiary sulphonium substances such as DMSP (dimethylsulphoniopropionate) or ectoine (1,4,5,6-tetrahydro-2-methyl-4-carboxylpyrimidine) (Ashraf and Harris 2004, Moghaieb *et al.* 2006, Lyon *et al.* 2011).

Synthesis and accumulation of osmolytes is by no means a characteristic of halophytes, but a general response of all organisms to any environmental condition leading to cellular dehydration (Yancey *et al*. 1982, Burg *et al.* 1996, Yancey 2005). Therefore, glycophytes also synthesise osmolytes when soil salinity increases, but it appears that they do not possess highly efficient mechanisms to transport toxic Na^+ and Cl^- ions into the vacuole, and that their – limited – resistance to salt stress is mostly dependent on the exclusion of salt at the root level (Munns and Tester 2008, Zhang *et al.* 2010, Kronzucker and Britto, 2011).

It is generally accepted that osmolytes are major contributors to maintaining the cellular osmotic balance under high salinity conditions; indeed, there are many reports on the accumulation of these compounds at relatively high cellular concentrations in different salt-tolerant plants (Parida and Das 2005, Flowers and Colmer 2008, and references therein). However, this may not always be the case as the concentration of organic, compatible solutes has been found to be much lower than that of inorganic ions – which would therefore be more important for osmotic adjustment – upon NaCl treatments of several halophytes; for example, in vetiver grass (*Vetiveria zizanioides*, Zhou and Yu 2009), quinoa (*Chenopodium quinoa*, Hariadi *et al.* 2011) or *Limonium latifolium* (Gagneul *et al.* 2007). Moreover, some results also suggest that osmolyte biosynthesis is only partially induced by salt because a large fraction of these compounds can already be stored in the cell before NaCl treatment; in *L.*

latifolium, application of salt stress slightly increased the contents of some compatible solutes, but also caused their redistribution between subcellular compartments (Gagneul *et al.* 2007). These data do not invalidate the hypothesis of compartmentalisation of toxic ions (Na^+ and Cl^-) in the vacuole and preferential accumulation of organic osmolytes – together with K^+, the non-toxic, physiological cation – in the cytosol, but call for more in-depth studies on the dynamics of the subcellular localisation of the different solutes contributing to osmotic adjustment under stress conditions.

Osmolytes as 'osmoprotectants'

Accumulation of compatible solutes in plants, in parallel with increased external salinity, has suggested, without demonstrating, a possible role of these compounds in salt tolerance mechanisms. Functional analyses could be carried out after identifying and cloning the genes responsible for the biosynthesis and catabolism of common osmolytes in different plant (and bacterial) species, which allows to manipulate their metabolism in transgenic plants to increase osmolytes' intracellular concentrations. Searching for an improvement of salt tolerance, several plant species – mostly *Arabidopsis thaliana* and tobacco, but there are also some examples with *Brassica napus* or rice – have been transformed with the appropriate genes. Indeed, enhanced resistance to high salinity – and/or to other abiotic stress conditions – has been generally observed, even though improvements were quite variable and often relatively modest (see, for example: Chen and Murata 2002, Borsani *et al.* 2003, Szabados *et al.* 2011, and references therein). These experiments, however, have challenged the 'classical' and accepted view that the primary role of compatible solutes is their contribution to osmotic adjustment: in many cases, either the concentration of the particular osmolyte in the transgenic plant was too low to possibly have any osmotic effect or there was no direct correlation between the increase in the levels of osmolytes and the stress tolerance improvements observed.

At present, it is clear that organic osmolytes play additional functional roles as 'osmoprotectants' in salt tolerance mechanisms (Ashraf and Foolad 2007, Iturriaga *et al.* 2009, Khan *et al.* 2010). Cellular dehydration, high ion concentrations and other stress conditions cause protein denaturation, and osmolytes may prevent it by helping to maintain the proper folding of proteins, acting as 'low-molecular-weight chaperons'. They can also interact directly with, and stabilise, multiprotein complexes, membranes and other cellular structures that are inactivated by stress (Singer and Lindquist 1998, Ignatova and Gierasch 2007, Holthauzen *et al.* 2011). There is substantial evidence to suggest that organic osmolytes also protect the cell against oxidative stress either as direct scavengers of ROS or by stabilising the antioxidant enzymes responsible for ROS elimination (Smirnoff and Cumbes 1989, Ashraf and Harris 2004, Jithesh *et al.* 2006). These compounds also constitute molecules for storage in the cell of C and/or N – and energy – to be used by the plant after stress conditions cease. Moreover, roles like signalling molecules involved in the regulation of gene expression and metabolic processes – which could be important for adaptation to or recovery from stress – have also been proposed for some osmolytes, such as proline (Szabados and Savoure 2010) or trehalose (Paul *et al*. 2008). Without doubt, this spells even more complexity for the possible functions of these compounds.

All these data reinforce the importance of osmolytes for salt tolerance, but they also complicate the analysis of the underlying mechanisms as their different functions cannot be easily separated. In any case, given these additional protective roles, osmolytes can significantly contribute to salt tolerance mechanisms even if, upon activation of their synthesis by salt treatments, they do not reach sufficiently high intracellular concentrations to have any substantial effect on osmotic adjustment.

Soluble carbohydrates as osmolytes in salt tolerance mechanisms

Many compatible solutes are secondary metabolites that are usually present in plant tissues at very low concentrations until their synthesis is activated under stress conditions. Accumulation of these compounds in response to high soil salinity or other abiotic stresses is, in fact, one of the criteria to define them as ‘osmolytes’. Nonetheless, the possibility in some cases that osmolytes are already present at significant levels in the absence of stress cannot be ruled out (see above). The situation is completely different for soluble sugars, such as sucrose, glucose or fructose. Direct products of photosynthesis and components of primary metabolism, sugars play several key roles in the cell: major energy sources, precursors of metabolic compounds and signalling molecules. Therefore, the intracellular concentrations of sugars must be regulated by complex mechanisms that control metabolic fluxes and signalling pathways, which makes it very difficult to assign them specific functions in the responses to salt stress. For example, an increase in the levels of, say, sucrose, in parallel to increasing external salinity might not be a primary response to salt stress, but the result of the reactivation of photosynthesis brought about by the activation of other defence mechanisms. In any case, if sugar contents reach significantly high levels, they will contribute to maintain cellular osmotic balance, and therefore to salt tolerance, irrespectively of why and how they accumulate in the cell. However, the presence of soluble carbohydrates at lower concentrations does not exclude a functional role in salt tolerance, mediated by their possible chaperon or ROS scavenging activities.

Bearing in mind this added complexity, there is still much evidence for the contribution of soluble carbohydrates to salt tolerance mechanisms in halophytes, as described for other types of compatible solutes. Sugars and polyols have been detected, at relatively high concentrations, in many halophytic taxa from different types of saline habitats. Many genes involved in the biosynthesis of soluble carbohydrates have been shown to be transcriptionally activated by salt and/or other abiotic stresses – although most of these data derive from experiments performed with salt-sensitive species. Some functional studies have also been done in transgenic plants in which an increased content of specific sugars or polyalcohols, by the overexpression of the appropriate genes, results in an enhancement of stress resistance – once again, using genes isolated generally from glycophytic plants and always transforming glycophytic model species, mostly *A. thaliana*, but also some crops. Accumulation of soluble carbohydrates has been determined in many different halophytes subjected to salt treatments under controlled laboratory or greenhouse conditions. There are also some – very few – field studies in which spatial or seasonal changes in carbohydrate contents have been measured and correlated with the degree of environmental stress of plants in their natural habitats.

In the following sections, some results of these studies are described and discussed, and those data supporting a functional role of soluble carbohydrates in salt tolerance are highlighted. Finally, details of the limitations and drawbacks of the experimental approaches commonly used to investigate these mechanisms are provided.

Detection and quantification of soluble carbohydrates in halophytic taxa

There are many published reports describing the chemical composition of different halophytes, specifically regarding quantitative analyses of inorganic and organic solutes – ions and compatible osmolytes – used by plants for osmotic adjustment in their natural saline habitats. These studies also attempted to establish whether particular osmolytes are exclusively or predominantly present in specific plant families and/or genera, and could be used as taxonomic criteria; halophytic taxa are widely distributed among angiosperms, and a correlation between the type of osmolyte used by different species and their taxonomic classification has been suggested. In the following paragraphs, we describe and comment on some early studies in which osmolyte contents were determined, in each case with a number of halophytes growing in the same habitat, thus allowing a comparison of the patterns obtained under the same environmental conditions in different species. A selection of these and other experimental data is shown in Table S1 (Supplementary Material), which includes only contents of sugars and polyols determined in different halophytes, but not the quantification of other osmolytes reported in the same references.

Monocotyledonous halophytes

In general, salt tolerance in monocotyledonous halophytes, when compared to their dicotyledonous counterparts, appears to be more dependent on the restriction of entry of inorganic ions into cells, the maintenance of higher cellular K^+/Na^+ ratios, and the preferential accumulation of soluble carbohydrates as osmotica (Albert and Popp 1978, Choo and Albert 1999, Gorham *et al*. 1980, Briens and Larher 1982). In most analysed species, sucrose was the sugar detected at higher cellular concentrations, although with extremely variable absolute values determined in different taxa, or even measured in the same taxon by different laboratories. Sucrose often represents more than 50% of total soluble sugars – sometimes even more than 80% – as described by Gorham *et al*. (1980) in *Carex extensa*, *C. arendaria*, *C. punctata*, *Scirpus maritimus*, *Juncus gerardii* or *J. maritimus*, or by Briens and Larher (1982) also in *Juncus maritimus* and *Scirpus maritimus*, as well as in *Phragmites communis*, *Agropyron pungens*, *Puccinellia maritima* or *Triglochin maritima*. Sometimes, however, other sugars such as glucose and/or fructose are detected at higher concentrations than sucrose, as found in *Puccinellia maritima*, *Agropyron pungens* and *Triglochin maritima* by Gorham *et al*. (1980), or in *Juncus gerardii* by Albert and Popp (1978). These results indicate that not only quantitative differences in the contents of specific carbohydrates have been observed for the same species, but also the relative patterns of accumulation of different sugars can be quite different. For example, in the leaves of *Agropyron pungens* collected from a salt marsh, Gorham *et al*. (1980) measured ca. 4-fold higher glucose contents than sucrose contents, while in the same species and in a similar sampling environment, a different salt marsh, Briens and Larher (1982) determined the presence of twice the levels of sucrose than glucose. Similar discrepancies as to other species (*Puccinellia maritima, Triglochin maritima*) are shown in these two reports. Apart from sucrose, glucose and fructose, other carbohydrates, such as the polyols pinitol or inositol, have been detected in some species, but they generally represent a minor contribution to the pool of total soluble carbohydrates (Gorham *et al*. 1980). It is also important to note that osmolyte contents can vary vastly in different plant organs; in those cases in which they have been analysed independently – quite often only leaf material is used for these measurements – generally higher levels of soluble carbohydrates have been found in roots, rhizomes or stems than in leaves; e.g., in *Juncus maritimus*, *Phragmites communis*, *Spartina townsendii* or *Triglochin maritime* (Briens and Larher, 1982). Yet there are also many species which do not follow this general trend, but present higher sugar contents in leaves, as shown by the same authors.

Despite the general preference for using carbohydrates as osmolytes, some monocots also accumulate other compatible solutes, such as proline, at even higher levels than those of total soluble sugars, as reported in *Triglochin maritima* (Briens and Larher 1982). In a more recent study, which does not include data on carbohydrates, Tipirdamaz *et al*. (2006) found high proline contents in several of the studied monocotyledonous halophytes, belonging to the families Cyperaceae (e.g., *Bolboschoenus maritimus*, *Cladium mariscus*), Poaceae (e.g., *Aeluropus littoralis*, *Polypogon monspeliensis*, *Puccinellia convoluta*, *P. distans*, and *P. koeieana*), and Liliaceae (e.g., *Allium atroviolaceum*).

Dicotyledonous halophytes

As opposed to monocots, dicotyledonous salt-tolerant plants usually show lower cellular K^+/Na^+ ratios, appear to be more efficient in storing toxic ions (Na^+ and Cl^-) at high concentrations in vacuoles, and maintain an osmotic balance by accumulating different types of osmolytes – amino acids, quaternary amines and/or soluble carbohydrates – in the cytoplasm (Albert and Popp 1978, Gorham *et al*. 1980, Briens and Larher 1982). Yet sugars seem less important for salt tolerance in dicots than in monocots. In line with this notion, it is frequent to find low levels of soluble carbohydrates in many species, especially in leaves, e.g., in *Camphorosma annua*, *Chenopodium glaucum* or *Lepidium crassifolium* (Albert and Popp 1978). However, as mentioned above for monocots, roots or stems can contain much higher levels of osmolytes than leaves; for example, sucrose contents below 100 µmol/g dry matter have been determined in the leaves of *Beta maritima*, *Halimione portulacoides* and *Limonium* *vulgare*, whereas in the roots of the same species, values of 1290, 655 and 966 µmol/g dry matter, respectively, have been obtained (Briens and Larher 1982).

Regardless of the presence or not of high concentrations of soluble sugars, dicotyledonous halophytes usually accumulate amino acids and quaternary ammonium compounds (e.g., proline and glycine betaine) under high salinity conditions, with some general trends observed in different families; for example, relatively high levels of amino acids, methylated onium and/or quaternary ammonium compounds have been detected in Amaranthaceae species (Gorham *et al*. 1980, Briens and Larher 1982, Tipirdamaz *et al*. 2006). Based on measurements taken in a large number of halophytes, it is generally assumed that species which behave as glycine betaine accumulators are poor proline accumulators, and *vice versa*. However, there are many exceptions to this rule: even within the same genus, there are proline accumulators and glycine betaine accumulators, but also species containing similar levels of both osmolytes (e.g., Tipirdamaz *et al*. 2006).

Unlike monocots, many halophytic dicots, particularly species living in mangrove habitats, contain relatively high levels of polyols. In most cases, mannitol, pinitol and inositol have been the most frequently detected compounds, while nitrogen-containing osmolytes usually accumulate at lower concentrations. Popp (1984) found pinitol to be the preferential osmolyte in the leaves of several Rhizophoraceae species (*Bruguiera exaristata*, *B. gymnorhiza*, *Ceriops tagal*, *Rhizophora apiculata*, *R. lamarckii*, *R. stylosa*) and mannitol in *Aegiceras corniculatum*, *Lumnitzera racemosa*, *Sonneratia alba*, and *Scyphiphora hydrophylacea*. Relatively high contents of several isomeric forms of inositol have been detected in different organs, such as twigs, roots or leaves, in *Rhizophora stylosa*, *Aegialitis annulata*, or *Melaleuca hyperacifolia* (Popp 1984, Popp and Polania 1989). Quebrachitol, an unusual polyol, has also been detected in *Excoecaria agallocha* (Popp 1984). Yet some exceptions have been reported in species belonging to the families Meliaceae (*Melia azedarach* and *Xylocarpus granatum*) and Picrodendraceae (*Micrantheum hexandrum*), in which significant concentrations of sucrose and reducing sugars (glucose and fructose) have been measured (Popp 1984).

To summarise, some general trends have been observed regarding differences in the types of solutes used by different halophytes for osmotic adjustment, mostly between mono- and dicotyledonous species. Yet these generalisations should be considered with caution since many exceptions have also been reported. What the above-mentioned results – and those included in Table S1 – clearly indicate is that a pattern of specific osmolytes accumulating in particular plant genera or families cannot be established, thus ruling out the possibility of their use for taxonomic classification. The only exception seems to be the Plantaginaceae family or, at least, the genus *Plantago*: in all the investigated species of the genus, the sugar-alcohol sorbitol has been identified as the physiological osmolyte (e.g., Ahmad *et al*. 1979, Konigshofer 1983, Gil *et al*. 2011). These data are in line with the idea that salt tolerance has appeared independently several times during angiosperm evolution (Flowers *et al*. 2010).

It is also evident the large qualitative and quantitative variability in osmolyte contents reported for different species – including related taxa of the same genus – and even for the same species, as reported by different laboratories. In our opinion, this variability is mostly due to the fact that the published data have been obtained from single samplings of plant material under specific environmental conditions, which differed in each particular study. In their natural habitats, halophytes are subjected to variable degrees of abiotic stress; for example, to short-term or seasonal changes in temperature, soil salinity or humidity, which affect osmolyte contents. Nonetheless, very few studies have aimed to determine ***changes*** in the levels of osmolytes in relation to plants' environmental conditions (see below). In any case, it is practically impossible to compare the huge amount of data published independently, which limits the informative value of all the experimental work described herein.

Functional analysis of soluble carbohydrates roles in salt stress responses

The enhancement of salt resistance of transgenic plants, when increasing the concentrations of different osmolytes by the overexpression of the enzymes involved in their biosynthesis, has provided valuable information on the protective roles of compatible solutes against salt stress, as discussed before. This functional approach is especially important in the case of soluble carbohydrates – sugars and polyols – because of their multiple metabolic and regulatory functions, which make it difficult to establish cause-effect relationships between salt treatments and changes in their intracellular levels. Nevertheless, alterations in the cellular contents of major sugars (sucrose, glucose or fructose) are expected to affect primary metabolism and have pleiotropic effects that could mask their possible roles as osmoprotectants. In fact, not many attempts have been made to modify the levels of these common sugars to improve stress resistance in transgenic plants. Despite this, we know that the possibility exists, as shown by Fukushima *et al.* (2001), who expressed a yeast invertase in the aploplast of transgenic tobacco; improved salt tolerance was observed in the GM plants, apparently due to the maintenance of high photosynthetic activity in the presence of salt.

Most experiments done in order to modify intracellular sugar contents have focused on the disaccharide trehalose. The presence of trehalose is not very common in plants (Ingram and Bartels 1996). Initially, it was described only in plants tolerant to desiccation, although more recently its accumulation under different abiotic stress conditions has been reported in other species (Fernandez *et al.* 2010, Deyanira *et al.* 2012). Several transgenic plants, transformed with trehalose biosynthetic genes, have been generated to investigate the function(s) of this sugar in stress responses. For example, the yeast trehalose-6-phosphate synthase gene (TPS1) has been expressed in tobacco (Holmström *et al.* 1996, Romero *et al.* 1997) and potato (Yeo *et al.* 2000), whereas the fused *E. coli* genes *otsA*, encoding the same synthase activity, and *otsB*, coding for trehalose-6-phosphate phosphatise, have been used to transform tobacco (Pilon-Smits *et al.* 1998) and rice (Garg *et al.* 2002). Increased trehalose contents in these transgenic lines correlated with improved resistance to drought, cold and/or high salinity; however, in general, trehalose levels remained too low to have any significant effect on osmoregulatory mechanisms. In addition, the constitutive expression of these genes generally caused multiple phenotypic alterations, including reduced growth and several developmental abnormalities, which were avoided in later experiments by the expression of the trehalose biosynthetic genes under the control of stress-induced promoters (e.g., Karim *et al.* 2007). Taken together, these results support a functional role for trehalose in salt stress resistance, which is probably not related to osmotic adjustment, but acts as a protective compound under cellular dehydration conditions, with chaperon and/or ROS scavenging activities. Not only do they suggested additional functions of trehalose as a signalling molecule involved in metabolic regulation, but also showed the need to tightly control its accumulation in transgenic plants to avoid the undesired side effects of altering carbohydrate metabolism.

Those side effects and developmental abnormalities were not observed when tampering with the intracellular levels of several sugar alcohols in transgenic plants. In fact, one of the first experiments to support a functional role of soluble carbohydrates – and of osmolytes, in general – in salinity tolerance mechanisms was the expression in transgenic *Nicotiana tabacum* plants of the *E. coli mt1D* gene, encoding mannitol-1-phosphate dehydrogenase, which led to increased levels of mannitol and improved salt tolerance, as compared to the non-transformed controls (Tarczynski *et al.* 1992, 1993). As mentioned before for trehalose, later work revealed that mannitol levels in transformed tobacco were too low to explain the observed enhancement of salt resistance based exclusively on osmotic adjustment, and an antioxidant function was proposed for this compound (Karakas *et al.* 1997). Similar results have been obtained through the expression of the same bacterial gene in other species, such as wheat (Abebe *et al.* 2003), *Pinus radiata* (Tang *et al.* 2005) or *Populus tomentosa* (Hu *et al.* 2005). In contrast, *A. thaliana* plants transformed with *mt1D* did not tolerate prolonged salt treatments, although their seeds were able to germinate in the presence of salt concentrations inhibitory for wild-type seeds (Thomas *et al.* 1995). As an alternative to the bacterial gene, Zhifang and Loescher (2003) engineered mannitol production in *Arabidopsis* by expression of the mannose-6-phosphate reductase gene isolated from celery under the control of the CaMV 35S promoter; the transformed adult plants presented substantially enhanced salt tolerance as they were able to complete their life cycle and to produce seeds in the presence of salt concentrations as high as 300 mM NaCl.

Phosphorylated derivatives of *myo*-inositol are essential signalling molecules in plants – in all eukaryotic organisms, actually – and are involved in multiple regulatory networks controlling plant development, metabolism and responses to biotic and abiotic stresses (e.g., Gillaspy 2011). *Myo*-inositol itself and methylated forms such as D-pinitol and D-ononitol are polyalcohols which, like mannitol, may play roles as osmolytes and osmoprotectants in plants, as suggested by the functional analyses of transgenic plants. For example, tobacco was transformed with the gene for *myo*-inositol-1-phosphate synthase (MIPS), the first enzyme in the biosynthetic pathway of *myo*-inositol from D-glucose-1-phosphate, isolated from *Porteresia coarctata*, a salt-tolerant species related to cultivated rice; transgenic plants showed *myo*-inositol accumulation in parallel with enhanced salt tolerance (Majee *et al.* 2004). The same improved salt resistance phenotype was observed upon the expression of this gene in rice (Das-Chatterjee *et al*. 2006). Similarly, overexpression in tobacco of the *imt1* gene – encoding *myo*-inositol *O*-methyltransferase – from the ice plant, *Mesembryanthemum crystallinum*, which led to accumulation of D-ononitol, also increased salt tolerance through enhanced photosynthetic activity in the transgenics, as compared to wild-type tobacco (Sheveleva *et al.* 1997). Ononitol content reached values of ca. 36 μmol/g FW in the leaves of the transgenic plants; assuming that the osmolyte is localised only in the cytoplasm, this would represent a cytosolic concentration of over 600 mM. In this case, therefore, the salt tolerance phenotype could be explained exclusively by the maintenance of the cellular osmotic balance in the presence of high external NaCl concentrations, independently of possible additional protective functions of D-ononitol. More recent experiments have demonstrated that the simultaneous expression in tobacco of the two previous genes – MIPS from *P. coarctata* and *imt1* from *M. crystallinum* – provided a greater degree of protection against salt stress than the individual expression of either gene since plants accumulated more total inositol and methylated inositol, grew better, displayed greater photosynthetic activity and were less prone to oxidative stress in the presence of salt (Patra *et al.* 2010). These experiments, by the way, represent some of the few examples of expression in transgenic plants of genes involved in carbohydrate metabolism isolated from halophytic species.

Another example of *in vivo* manipulation of sugar-alcohols levels is the generation of transgenic persimmon (*Diospyros kaki*) plants overexpressing the apple sorbitol-6-phosphate dehydrogenase gene; GM plants showed increased levels of sorbitol, which, once again, correlated with enhanced resistance to salt stress (Gao *et al.* 2001).

From a biotechnological point of view, the results mentioned above support the feasibility of improving salt tolerance in transgenic crops by engineering osmolyte metabolism to increase the intracellular levels of specific compatible solutes; they also indicate that the best approach is the regulated and coordinated expression of several appropriate genes under the control of stress-induced promoters. It remains to be seen if these genetic modifications will affect the yield and other agronomic characteristics of the GM crops.

On the other hand, if an increase in the cellular content of a particular sugar or polyol is sufficient to improve the response to NaCl stress of salt-sensitive species, such as tobacco or *Arabidopsis*, be it to a greater or lesser extent, it would seem logical to assume that accumulation of high levels of the same compound under natural stressful conditions may also contribute to tolerance in salt-tolerant species. However, it is not known if the results obtained with those genetically modified plants can be extrapolated to the stress responses of halophytes in their natural habitats; all these studies provide only indirect support to the possible functional role of soluble carbohydrates in tolerance mechanisms to high soil salinity in halophytes.

Salt stress-induced expression of genes involved in carbohydrate metabolism

Transcriptional activation of a specific plant gene under high salinity conditions is generally considered evidence for its participation in plant responses to salt stress. Yet this is not necessarily true since induction of gene expression could be a secondary effect that is not directly related to the stress response. Many of the genes involved in the biosynthesis of soluble carbohydrates in salt-sensitive plants have been shown to be activated by salt. Unfortunately, there are very few studies available on the regulation of the same metabolic pathways in halophytes; for example, those of Bohnert and co-workers in *Mesembryanthemum crystallinum*, showing that the genes encoding *myo*-inositol 1-phosphate synthase (Ishitani *et al*. 1996) and *myo*-inositol *O*-methyl transferase (Vernon and Bohnert, 1992a,b), responsible for the first steps in the synthesis of *myo*-inositol and pinitol, respectively, are both activated under salt stress conditions. It is interesting to note that this pathway is not regulated by salt in *Arabidopsis*, and is an example of the differences between salt-sensitive and salt-tolerant plants in terms of induction of osmolyte biosynthesis. The *myo*-inositol *O*-methyl transferase gene, which is not present in the genome of cultivated rice, is also up-regulated by salt in the halophytic wild rice *Porteresia coarctata*, leading to accumulation of pinitol (Sengupta *et al*. 2008).

In recent years, genome-wide analyses of gene expression have also been performed in some halophytes to detect the genes that are transcriptionally activated upon treatment of the plants with NaCl by different techniques: construction of specific cDNA libraries and ESTs identification, subtractive hybridisation or transcriptomic analysis. Among the genes expressed at higher levels in the presence of salt, several involved in the synthesis of soluble carbohydrates with presumed osmolyte functions have been identified. To name but a few, there are those encoding *myo*-inositol 1-phosphate synthases from *Thellungiella salsuginea* (formerly *T. halophila*) (Taji *et al*. 2004) and *Spartina alterniflora* (Baisakh *et al*. 2008), mannose 6-phosphate reductase from *Tamarix hispida* (Li *et al*. 2009), or myo-inositol oxygenase from *Puccinellia tenuiflora* (Wang *et al*. 2007), along with many genes encoding enzymes of general carbohydrate metabolism, whose enhanced expression could affect the levels of different soluble sugars. Proteomics, used to identify salt-induced proteins in some halophytes, such as *Porteresia coarctata* (Sengupta and Majumder 2009) or *Puccinellia tenuiflora* (Yu *et al*. 2011), have also allowed the detection of several proteins putatively involved in carbohydrate metabolism. Nevertheless, there are still relatively few examples of these technologies having been applied to the study of salt stress responses in halophytic species since genomic and proteomic analyses of salt stress responses have focused mostly on glycophytic models, as when using more traditional methods.

Accumulation of soluble carbohydrates in halophytes upon controlled salt treatments

As mentioned in the Introduction, most studies on plant responses to salinity have been carried out in glycophytes, but there are still many reports describing the physiological and biochemical changes observed in different halophytes subjected to specific salt stress treatments in the laboratory or the greenhouse. The parameters determined vary considerably, and may include growth measurements of shoots and/or roots, photosynthesis activity and photosynthetic pigments contents, water relations in the plants, enzyme activities – e.g., of antioxidant systems – or levels of different ions and compatible solutes. Among osmolytes, nitrogen-containing compounds, such as proline and glycine betaine, are often quantified, but data on sugars and/or polyols contents, for either specific compounds or merely as 'total soluble carbohydrates', are also included in some papers. In the following paragraphs, some of these published data are briefly commented on (additional examples are included in Table 1).

Salt-stress treatments of halophytes often correlate with an increase in total soluble carbohydrate contents in the plants. For example, in *Kochia prostrata* [synonym of *Bassia prostrata*] (Amaranthaceae) seedlings grown for 30 days in the presence of increasing NaCl concentrations, up to 200 mM (Karimi *et al.* 2005); in this case, plant growth was only slightly reduced to below 150 mM NaCl, but was significantly inhibited by the highest salt concentration tested; in parallel, soluble sugar contents progressively rose to double at 150 mM NaCl, and an increase of more than 5-fold at 200 mM NaCl was recorded, if compared to the level in the non-stressed control seedlings. The same qualitative pattern was observed for accumulation of proline and glycine betaine, suggesting that nitrogen-containing osmolytes can contribute, together with carbohydrates, to the salt tolerance mechanisms in this species (Karimi *et al.* 2005). Similar results have been obtained in the roots and leaves of *Vetiveria zizanioides* [synonym of *Chrysopogon zizanioides*] (Poaceae) seedlings treated with salt for 9 days, although the observed increases in total soluble sugar contents were relatively lower, below 2-fold in both organs at the highest concentration used, 300 mM NaCl (Zhou and Yu 2009). In this last example, the levels of soluble sugars reached under the strongest stress conditions were similar in the roots and leaves of vetiver grass seedlings, about 200 and 300 μmol/g dry weight, respectively. However, there are also reports showing completely different patterns of sugar accumulation in different organs in response to salt treatments of the plants; thus, in *Aster tripolium* (Asteraceae) plants irrigated at different salinity levels, significant increases of soluble carbohydrate contents were detected only in the main roots, they decreased in lateral roots, and no changes were observed in either old or young leaves; in contrast, proline levels substantially increased in both leaves and main roots (Geissler *et al.* 2009). Apart from seedlings or young plants, plant tissue culture material has also been used to assess the responses of halophytes to salinity stress, as described, for example, by Lokhande *et al.* (2011) for axillary shoots induced from the nodal explants of *Sesuvium portulacastrum* (Aizoaceae), a mangrove-associated halophyte. Treatments of shoots for 30 days with up to 600 mM NaCl resulted in increased levels of soluble sugars; however, maximum contents were determined at 200 mM sodium chloride, which represents the optimal salt concentration for this material, as shown by measurements of several growth-related parameters. Conversely, proline and glycine betaine levels were at their lowest under these conditions, although they rose at higher salt concentrations: 400 and 600 mM NaCl. Considering these data, it is likely in this case that increased sugar levels is not a response to elevated salinity, rather a reflection of a more active carbohydrate metabolism related to the optimal growth conditions of plant material.

In most studies, including all those cited above, NaCl was the salt used for stress treatments but, in nature, saline soils are often also alkaline due to the presence of additional ions. To investigate the interactive effects of these two stresses on *Spartina alterniflora* (Poaceae), Li *et al.* (2010) treated 4-week-old seedlings for 2 weeks with several combinations of sodium salts, neutral and alkaline to obtain different salinity levels combined with distinct pH values. At a neutral pH, no accumulation of soluble carbohydrates was detected below 200 mM salt, but their level increased with raised salinity up to ca. 2.5-fold at 600 mM, and also with increased pH for each fixed salinity level. A similar pattern of variation was also observed for proline contents. Therefore, reciprocal enhancement appears between salt and alkali stress, at least in this species.

Salt stress treatments of plant material are usually designed to determine concentration-dependent changes in different parameters – such as sugars and polyols contents – that is, the plants are maintained for a fixed time in the presence of different salt concentrations. Kurkova *et al.* (2002) used an alternative approach to analyse the responses of *Seidlitzia rosmarinus* [synonym of *Salsola schweinfurthii*] (Amaranthaceae) to salt stress: a ‘shock treatment’ with 500 mM NaCl was applied to two-month-old plants and several measurements were carried out at different times during the following 72 hours, including those of sucrose contents in leaves and roots, which increased in both organs (2.4-fold and 1.5-fold, respectively) during the first 60 min of treatment, to decrease again later to values close to, or even below, those determined at time zero. Ruffino *et al.* (2010) also studied time-dependent responses at a single salt concentration, but within a more extended time frame; *Chenopodium quinoa* (Amaranthaceae) seeds were germinated in the presence of 250 mM NaCl, and sugar contents were determined in the seedlings cotyledons after 6, 12 and 21 days. A time-dependent increase of total soluble sugars, sucrose, fructose and glucose was detected in both salt-treated seedlings and untreated controls; while the increase in total sugars and glucose was relatively higher in the presence of NaCl, no differences were observed for sucrose or fructose.

The behaviour of different, non-related plant species regarding the use of soluble carbohydrates as osmolytes, can prove completely different, as shown by the previous examples. It is, therefore, especially interesting to compare the responses of related taxa, e.g., different species of the same genus, when subjected to the same stress treatments, as reported by Orlova *et al.* (2009) for two *Artemisia* (Asteraceae) species: *A. lerchiana* and *A. pauciflora*. The seedlings of both taxa responded in a similar way, qualitatively, to increasing external NaCl concentrations, with a parallel increase in the accumulation of the trisaccharide raffinose and a drop in the levels of other sugars (sucrose + trehalose, glucose, fructose and sorbose) noted in both leaves and roots. Quantitatively, however, clear differences were detected between the two species as the increases in raffinose contents were much higher in *A. pauciflora* than in *A. lerchiana*: 5.5-fold *vs*. 1.4-fold and 9.2-fold *vs*. ca. 3-fold, in leaves and roots, respectively.

A completely different pattern of variation of sugar and polyol contents was observed when comparing several species of the genus *Limonium* (Plumbaginaceae) – *L. latifolium* (Gagneul *et al*. 2007), *L. perezii* and *L. sinuatum* (Liu and Grieve 2009) – whose responses to salt treatments were generally not even qualitatively similar. Thus, in *L. latifolium*, a rise in sucrose, fructose and glucose contents was noted with increasing salinity; in *L. perezii*, glucose and fructose increased while sucrose decreased; and in *L. sinuatum*, the levels of the two monosaccharides remained more or less constant, while sucrose contents slightly increased. Perhaps the only relevant common features observed in the three species were the low levels of *myo*-inositol, which did not vary in response to salt stress, and the presence of a not very common isomer, *chiro*-inositol, which accumulated to sufficiently high levels, albeit quite distinct for different taxa, to significantly contribute to osmotic adjustment. Nevertheless, these authors did not detect pinitol in the above-mentioned *Limonium* species; this common stress-induced cyclitol was identified, however, as a prominent carbohydrate in field-collected material of *L. gmelinii* ssp. *hungarica* (Murakeözy *et al*. 2002).

These examples, together with those shown in Table 1, along with many more data from the literature, indicate that salt treatments indeed modify carbohydrate metabolism in all the investigated halophytes, and sometimes lead to the concentration-dependent accumulation of specific sugars or polyalcohols, which may reach sufficiently high levels to have a significant effect on osmotic adjustment. In such cases, the particular carbohydrate is likely to contribute to salt tolerance under the experimental conditions used, although the induction of its synthesis may not be a direct response to the salt treatment, but a secondary one to other defence reactions. However, these stress treatments are not standardised, and extremely different experimental conditions have been used in several studies; in addition, salt stress has been applied to a variety of plant materials, e.g., germinating seeds, seedlings or young plants, but rarely to adult plants, and it is well-known that salt stress responses depend largely on the developmental stage of plants (Johnson *et al*. 1992, Vicente *et al*. 2004, Grigore *et al*. 2012). For these reasons, it is not possible to draw any general conclusions from all the data published independently, except to confirm the wide variability in the responses to salt stress observed in different species, with no clear, quantitative or qualitative, general patterns of accumulation of specific sugars or polyalcohols.

Environmentally induced changes of soluble carbohydrates contents in halophytes

The responses of a particular halophytic species to salt stress under controlled artificial conditions – in general, but also specifically regarding the accumulation of compatible solutes – may differ considerably from its behaviour in nature, where plants must react not only to soil salinity, but simultaneously to a combination of environmental stresses, which will continuously change in unpredictable ways, probably affecting their osmolytes contents. Moreover, it is difficult to establish the relative contribution of different stress responses to stress tolerance in a given tolerant species.

Fieldwork is necessary to assess the biological relevance of the accumulation of soluble carbohydrates for salt tolerance in halophytes – or, more precisely, abiotic stress tolerance in general since, as mentioned above, in nature salt stress cannot be considered independently of other stressful environmental conditions. Therefore, as a complementary approach to the studies described before, changes in the levels of soluble carbohydrates can be determined in plants growing in their natural habitats, and the degree of environmental stress affecting plants can be estimated in parallel by measuring soil properties, such as electrical conductivity, ion contents and humidity, and recording meteorological parameters: e.g., temperature, rainfall or UV irradiation. A positive correlation between an increased level of abiotic stress and the accumulation of specific sugars or polyols would represent direct evidence for their contribution to tolerance mechanisms, but under natural ecologically relevant conditions; obviously, this strategy can be extended to other types of osmolytes and, in general, to any biochemical marker of plant stress responses.

Very few published studies exist in which soluble carbohydrates contents have been determined using this experimental approach in halophytes of the same species, but growing in different locations. For example, Youssef (2009) compared responses to salinity in five succulent halophytes (*Halocnemum strobilaceum*, *Arthrocnemum macrostachyum*, *Halopeplis perfoliata*, *Suaeda vermiculata* and *Seidlitzia rosmarinus*) collected from two coastal sites along the Arabian Gulf in Saudi Arabia (site 1), and at the Red Sea in Egypt (site 2). Among many other measurements, total soluble sugars were determined and, for all five species, higher values were obtained for those collected at site 1, where environmental conditions appeared to be more stressful for plants: higher electrical conductivity, total soluble salts and ions contents and lower water content in soil, as well as higher average temperatures. In a similar, more recent study, Bankaji and Sleimi (2012) analysed *Salicornia arabica*, *Suaeda fruticosa*, *Atriplex portulacoides* and *A. halimus* plants collected from three different localities in north Tunisia; for each species, they found a positive correlation between the soluble sugar contents in plants and the degree of environmental stress in the respective habitats, estimated from meteorological data and soil electrical conductivity measurements. However in this kind of experiments, the possibility that differences in sugar contents are due to genetic variability between the populations of each species growing in different habitats and adapted to their specific conditions, rather than to an induced response, cannot be ruled out.

To avoid this possibility, an alternative approach would be to determine seasonal variations in carbohydrates contents in the same plant population present in a given location. This was done, for example, by Doddema *et al.* (1986) who, when investigating the effects of seasonal changes of soil salinity on the metabolism of *Arthrocnemum fruticosum* in a saline area by the Dead Sea in Jordan, found a sudden and temporary increase in total soluble carbohydrate contents in plant roots in the month of June. This increase was about 6-fold more than in previous months, and was accompanied by an abrupt increase in soil salinity and a rise in sodium contents in plants. In *Limonium gmelini* subsp. *hungarica* growing in an inland saline grass area in Hungary, Murakeözy *et al*. (2002) determined soluble carbohydrate levels in plant material collected at several time points throughout the year. Among other data, a 5-fold increase in the level of leaf pinitol, identified as a mayor osmolyte in this taxon, was observed in summer as compared to the lowest values recorded in mid-April, and additional peaks were obtained in winter/early spring; the higher pinitol contents corresponded to the periods of more intense stress due to reduced rainfall and lower soil water content, and to lower temperatures, respectively. These studies were extended by the same group to two other halophytes present in the same habitat, *Lepidium crassifolium* and *Camphorosma annua*, and also included measurements of soil electrical conductivity (Murakeözy *et al*. 2003); as a general pattern, and despite the clear quantitative differences among the three taxa, the highest levels of reducing sugars, sucrose and pinitol were detected in early spring and correlated with maximum soil salinity and the lowest atmospheric temperature.

Sometimes, changes in osmolytes levels have been related to environmental factors other than soil salinity. By way of example, in a study on subantarctic Kerguelen cabbage (*Pringlea antiscorbutica*), Aubert *et al.* (1999) found correlations between levels of glucose, the major sugar in leaves, and annual irradiance, and between starch content in stems and roots and daily air temperature. Walker *et al*. (2008) determined seasonal changes in the cold-tolerance of *Atriplex halimus* plants grown in the field at two sites with markedly different average minimum temperatures, and established a positive correlation with the concentration of soluble sugars in leaves, among other factors. More recently, Mouri *et al.* (2012) detected significant seasonal variations in total soluble sugars and proline contents in *Ammophila arenaria* from sand dunes in Algeria, both increasing in summer and autumn, as compared with winter and spring. The authors accounted for these high values with the intense drought and high temperatures affecting plants in the two seasons, but did not present experimental measurements to confirm their statement.

Finally, we refer to a systematic study on the seasonal variation of soluble carbohydrate contents carried out by our group in five perennial halophytes growing in a littoral salt marsh near the city of Valencia (E Spain) (Gil *et al.* 2011). Plant material was collected in five successive samplings, from summer 2009 to autumn 2010, from the same individual plants of each analysed species, to determine the levels of major soluble carbohydrates. Analyses of soil samples taken simultaneously to plant material, together with recorded meteorological data, were used to assess the level of environmental stress affecting the plants in the experimental plot. Summer 2009 was the most stressful period with the highest values for soil electrical conductivity, atmospheric temperature, evapotranspiration and water deficit due to absolute lack of rain during the month prior to sampling. In summer 2010, soil salinity was slightly lower and average temperatures were similar, but it was not as dry as summer 2009. Spring 2010 was the mildest season, with the lowest level of soil salinity and a lot of rain. Concerning osmolyte contents, sorbitol was the only carbohydrate detected at significant levels in *Plantago crassifolia*; most importantly, a very good correlation was found between sorbitol contents and the degree of environmental stress, with an almost 6-fold difference between summer 2009 and spring 2010; very low levels were detected for the rest of sugars and polyols, with no correlation with environmental conditions. These findings support the idea that sorbitol is the physiologically relevant osmolyte in this species, as is believed to be the case for all Plantaginaceae taxa (Flowers *et al.* 2010). The other two dicotyledonous halophytes included in the study, *Inula crithmoides* and *Sarcocornia fruticosa*, are typical glycine betaine accumulators (Boscaiu *et al.* 2011) and contain excessively low levels of soluble carbohydrates to contribute significantly to osmotic adjustment; in addition, no clear correlation of sugar or polyol levels was found with the degree of abiotic stress, except for glycerol in *I. crithmoides*, which could contribute to salt tolerance in this species due to its putative 'osmoprotectant' function. The study also included two monocotyledonous halophytes, *Juncus maritimus* and *J. acutus*, two closely related species; both accumulated relatively high levels of sucrose and, to a lesser extent, glucose and fructose – all three sugars could substantially contribute to osmotic balance – but showed very low polyol contents. In *J. acutus*, seasonal variations were statistically significant, with the highest levels detected in summer 2009 for sucrose (ca. 150 µmol g^{-1} DW), glucose and fructose (ca. 65 µmol g^{-1} DW each). Sugar accumulation patterns were quantitatively and quantitatively similar in the more salt-tolerant *J. maritimus*, but seasonal variations were slighter and not significant. Nevertheless, for both taxa a positive correlation between seasonal changes in sugar contents and soil/climatic conditions associated with salt and water stress were established by the principal component analysis (PCA) statistical method (Gil *et al*. 2011).

We believe that the experimental approach followed in this and similar studies can provide novel and interesting information on the biological function of osmolyte accumulation and its relevance for stress tolerance of specific halophytes in their natural habitats, thus extending and complementing the work carried out in the laboratory, under controlled stress conditions, with either glycophytic models or salt tolerant species.

Conclusions and perspectives

The cellular accumulation of soluble carbohydrates – or other compatible solutes – is well established as a general response of plants to salt stress. As discussed above, there are also many indirect evidences for the actual contribution of sugar and polyols to salt tolerance of specific halophytic taxa, mostly based on the concentration- or time-dependent increases in osmolyte levels that have been observed upon treatment of the plants with NaCl. In the specific case of sugars, their accumulation in parallel with increasing external salinity may not be a direct response to salt stress, but a secondary effect of a general stimulation of carbohydrate metabolism resulting from other primary responses. Nevertheless, if sugars reach concentrations high enough to substantially contribute to osmotic adjustment, most likely they will also contribute to salt tolerance; however, low carbohydrate levels do not rule out a functional role of these compatible solutes in salt tolerance mechanisms, role which would depend on their additional activities as low-molecular-weight chaperons and/or ROS scavengers. Another general conclusion of the published work presented here, is the necessity of assessing the responses to salt stress on a case-by-case basis, because of the huge variability observed, quantitatively as well as qualitatively, in the patterns of soluble carbohydrate accumulation in different halophytic species – although this variability could be partly due to the disparate experimental conditions used in different studies. In any case, it is not clear whether the results of experiments carried out in the laboratory can be extrapolated to the behaviour of plants in nature.

Concerning methodological aspects, many data on osmolyte contents have been obtained using ‘classical’ chemical or enzymatic assays based on espectrophotometric measurements. In the last years, new metabolic profiling technologies – for example, gas chromatography coupled to mass spectrometry, HPLC also coupled to MS or to different types of detectors, Fourier-transformed infrared spectroscopy or NMR-based methods – have been applied to the study of plant responses to stress, generally in salt-sensitive models, but also in a few halophytes (Sanchez *et al*. 2008). These studies included determination of osmolyte levels in field-collected material and also analysis of salt-induced changes in osmolyte patterns upon salt treatments of the plants (e.g., Murakeözy *et al*. 2003, Tipirdamaz *et al*. 2006, Gagneul *et al*. 2007, Alla *et al*. 2012).

For all the reasons discussed above, we think that more effort should be invested on fieldwork, and propose to step up research on halophytes in their natural ecosystems, correlating seasonal changes in soluble carbohydrates contents with the degree of environmental stress affecting the plants. Metabolomics should be used in these comparative analyses, which would obviously include, along with compatible solutes, other metabolites possibly involved in abiotic stress tolerance mechanisms. We also propose to extend this kind of comparative studies to the analysis of stress responses in closely related species of the same genus, but showing different degrees of salt tolerance. *Plantago* and *Juncus* are examples of dicot and monocot genera, respectively, appropriate for these studies as both include taxa with a wide range of salt sensitivity, from typical glycophytes to highly salt-tolerant species. The same line of work, based on the comparison of metabolic profiles, is been successfully used with the salt-sensitive *Arabidopsis thaliana* and stress-tolerant species, such as *Tellungiella salsuginea*, considered also as ‘close relatives’, although they belong to different genera and have less than 80% overall genomic homology (e.g., Gong *et al*. 2005). We believe that these strategies, complementary to more common approaches based on the use of glycophytic models, will provide novel information that will contribute to improve and broaden our knowledge about salt tolerance mechanisms… and that will help to answer the question put forward in the title of this review.

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Table S1. Relevant concentrations of soluble carbohydrates in halophytes under natural saline conditions.

Concentration data of carbohydrates were obtained from tables or graphs and expressed in dry weight – µmol g^{-1} DW – or fresh weight – mol m^{-3} plant water – according to authors. Carbohydrate abbreviations; Suc, sucrose; Glu, glucose; Fru, fructose; Ino, inositol – Chiro-i, *chiro*-inositol; Muco-i, *muco*-inositol; Myo-i, *myo*-inositol; Scy-i, *scyllo*-inositol –; Man, mannitol; Pin, pinitol; Que, Quebrachitol.

Species	Habitat	Organ	CHO	Conc.	Units	Reference
Monocotyledoneae						
Cyperaceae: *Bolboschoenus maritimus* (L.) Palla [= *Scirpus maritimus* L.]	Salt marsh	Leaves	Suc	185	µmol g^{-1} DW	Briens and Larher 1982
			Glu	21		
			Fru	25		
		Rhizomes	Suc	342		
			Glu	21		
			Fru	20		
		Roots	Suc	89		
			Glu	28		
			Fru	26		
		Leaves	Suc	58.2	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	3.5		
			Fru	6.3		
			Ino	6.2		
	Saline lake		Suc	~140		Albert and Popp 1978
			Glu	~40		
			Fru	~40		
			Ino (Myo-i)	8		
Carex distans L.	Saline lake	Leaves	Suc	~150	mol m^{-3} PW	Albert and Popp 1978
			Glu	~50		
			Fru	~25		
Carex duriuscula C.A.Mey.	Semi-arid salt-alkalinized grassland	Shoots	Man	29.7	µmol g^{-1} DW	Yang *et al.* 2012
Carex extensa Gooden.	Salt marsh	Leaves	Suc	121.8	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	11		

			Fru	5.6		
Carex punctata Gaudin	Salt marsh	Leaves	Suc	114.1	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	7.9		
			Fru	4.9		
			Ino	6.8		
			Pin	8.9		
Juncaceae: *Juncus articulatus* L.	Salt marsh	Leaves	Suc	17	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	6.8		
			Fru	8.9		
Juncus gerardii Loisel.	Salt marsh	Leaves	Suc	90.4	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	7.2		
			Fru	40.5		
	Saline lake		Suc	~10		Albert and Popp 1978
			Glu	~75		
			Fru	~75		
Juncus maritimus Lam.	Salt marsh	Leaves	Suc	171	µmol g^{-1} DW	Briens and Larher 1982
			Glu	21		
			Fru	20		
		Rhizomes	Suc	515		
			Glu	100		
			Fru	105		
		Roots	Suc	216		
			Glu	27		
			Fru	31		
		Leaves	Suc	79.9	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	18.1		
			Fru	24.9		
Juncaginaceae: *Triglochin maritima* L.	Salt marsh	Leaves	Suc	151	µmol g^{-1} DW	Briens and Larher 1982
			Glu	63		
			Fru	82		

		Roots	Suc	326		
			Glu	17		
			Fru	22		
		Leaves	Suc	8.2	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	42.8		
			Fru	34.8		
			Ino	2		
	Saline lake		Suc	~2		Albert and Popp 1978
			Glu	~75		
			Fru	~75		
Iridaceae: *Iris pseudacorus* L.	Salt marsh	Leaves	Suc	16.3	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	12.8		
			Fru	11.7		
			Ino	4.8		
Poaceae: *Agrostis stolonifera* L.	Saline lake	Leaves	Suc	~40	mol m^{-3} PW	Albert and Popp 1978
			Glu	~40		
			Fru	~50		
			Ino (Myo-i)	4		
Calamagrostis epigejos (L.) Roth [= *Calamagrostis macrolepis* Litv.]	Semi-arid salt-alkalinized grassland	Shoots	Man	40.8	µmol g^{-1} DW	Yang *et al.* 2012
Chloris virgata Sw.	Semi-arid salt-alkalinized grassland	Shoots	Man	35.1	µmol g^{-1} DW	Yang *et al.* 2012
Crypsis aculeata (L.) Aiton	Saline lake	Leaves	Suc	~90	mol m^{-3} PW	Albert and Popp 1978
			Glu	~30		
			Fru	~50		
			Ino (Myo-i)	4		
Elymus pungens (Pers.) Melderis [= *Agropyron pungens* (Pers.) Roem. & Schult.]	Salt marsh	Leaves	Suc	80	µmol g^{-1} DW	Briens and Larher 1982
			Glu	38		
			Fru	25		

		Roots	Suc	46		
			Glu	14		
			Fru	16		
		Leaves	Suc	10	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	43		
			Fru	17.5		
Festuca rubra L.	Salt marsh	Leaves	Suc	126	µmol g^{-1} DW	Briens and Larher, 1982
			Glu	88		
			Fru	78		
		Roots	Suc	65		
			Glu	22		
			Fru	34		
Leymus chinensis (Trin.) Tzvelev	Semi-arid salt-alkalinized grassland	Shoots	Man	27.8	µmol g^{-1} DW	Yang *et al.* 2012
Phalaris arundinacea L.	Salt marsh	Leaves	Suc	17.6	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	10.6		
			Fru	9.4		
Phragmites australis (Cav.) Trin. ex Steud. [= *P. communis* Trin.] [= *P. hirsuta* Kitag.]	Salt marsh	Leaves	Suc	236	µmol g^{-1} DW	Briens and Larher 1982
			Glu	67		
			Fru	80		
		Stems	Suc	404		
			Glu	32		
			Fru	43		
		Roots	Suc	121		
			Glu	30		
			Fru	33		
	Saline lake	Leaves	Suc	~70	mol m^{-3} PW	Albert and Popp 1978
			Glu	~55		
			Fru	~50		
	Semi-arid salt-alkalinized	Shoots	Man	27.4	µmol g^{-1} DW	Yang *et al.* 2012

	grassland					
Puccinellia distans (Jacq.) Parl.	Saline lake	Leaves	Suc	~110	mol m^{-3} PW	Albert and Popp 1978
			Glu	~65		
			Fru	~80		
Puccinellia maritima (Huds.) Parl.	Salt marsh	Leaves	Suc	217	µmol g^{-1} DW	Briens and Larher 1982
			Glu	20		
			Fru	49		
		Roots	Suc	60		
			Glu	20		
			Fru	22		
		Leaves	Suc	39.3	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	37.7		
			Fru	93.9		
Puccinellia tenuiflora (Griseb.) Scribn. & Merr.	Semi-arid salt-alkalinized grassland	Shoots	Man	38.1	µmol g^{-1} DW	Yang *et al.* 2012
Spartina anglica C.E.Hubb.	Salt marsh	Leaves	Suc	17.2	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	4.8		
			Fru	13.2		
			Ino	0.6		
Spartina x townsendii H.Groves & J. Groves	Salt marsh	Leaves	Suc	167	µmol g^{-1} DW	Briens and Larher 1982
			Glu	20		
			Fru	92		
		Roots	Suc	620		
			Glu	103		
			Fru	231		
Dicotyledoneae						
Acanthaceae: *Acanthus ilicifolius* L.	Mangrove	Leaves	Suc	~15	mol m^{-3} PW	Popp 1984
Avicennia marina (Forssk.) Vierh.	Mangrove	Leaves	Suc	~30	mol m^{-3} PW	Popp 1984
Amaranthaceae: *Atriplex portulacoides* L.	Salt marsh	Leaves	Suc	50	µmol g^{-1} DW	Briens and Larher 1982

[= *Halimione portulacoides* (L.) Aellen]			Glu	23		
			Fru	41		
		Stems	Suc	238		
			Glu	30		
			Fru	20		
		Roots	Suc	655		
			Glu	55		
			Fru	54		
Atriplex prostrata Boucher ex DC. subsp. *calotheca* (Rafn) M.A.Gust. [= *Atriplex hastata* auct., non L.]	Salt marsh	Leaves	Suc	75	µmol g^{-1} DW	Briens and Larher 1982
			Glu	15		
			Fru	10		
		Stems	Suc	60		
			Glu	651		
			Fru	150		
		Roots	Suc	147		
			Glu	107		
			Fru	54		
	Saline lake	Leaves	Suc	~5	mol m^{-3} PW	Albert and Popp 1978
			Glu	~3		
			Fru	~2		
			Pin	7.6		
Bassia scoparia (L.) A.J.Scott [= *Kochia sieversiana* (Pall.) C.A. Mey.]	Semi-arid salt-alkalinized grassland	Shoots	Man	18.5	µmol g^{-1} DW	Yang *et al.* 2012
Beta vulgaris L. [= *Beta maritima* L.]	Salt marsh	Leaves	Suc	97	µmol g^{-1} DW	Briens and Larher 1982
			Glu	157		
			Fru	90		
		Stems	Suc	295		
			Glu	194		
			Fru	9		
		Roots	Suc	1290		
			Glu	96		

			Fru	75		
Camphorosma annua Pall.	Saline lake	Leaves	Suc	~2	mol m^{-3} PW	Albert and Popp 1978
			Glu	~30		
			Fru	~15		
			Pin	3.8		
Chenopodium chenopodioides (L.) Aellen [= *Chenopodium botryoides* Sm.]	Saline lake	Leaves	Suc	~5	mol m^{-3} PW	Albert and Popp 1978
			Glu	~20		
			Fru	~35		
Chenopodium glaucum L.	Saline lake	Leaves	Suc	~10	mol m^{-3} PW	Albert and Popp 1978
			Glu	~15		
			Fru	~15		
Salicornia europaea L.	Salt marsh	Leaves	Suc	27	µmol g^{-1} DW	Briens and Larher 1982
			Glu	16		
			Fru	15		
		Stems	Suc	86		
			Glu	5		
			Fru	4		
		Roots	Suc	109		
			Glu	15		
			Fru	12		
		Leaves	Suc	12.8	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	4.6		
			Fru	9.4		
Salicornia prostrata Pall.	Saline lake	Leaves	Suc	~5	mol m^{-3} PW	Albert and Popp 1978
			Glu	~10		
			Fru	~20		
Suaeda glauca (Bunge) Bunge	Semi-arid salt-alkalinized grassland	Shoots	Man	26.4	µmol g^{-1} DW	Yang *et al.* 2012
Suaeda macrocarpa Moq.	Salt marsh	Leaves	Suc	68	µmol g^{-1} DW	Briens and Larher 1982

			Glu	7		
			Fru	5		
		Stems	Suc	35		
			Glu	7		
			Fru	6		
		Roots	Suc	97		
			Glu	8		
			Fru	10		
Suaeda maritima (L.) Dumort.	Salt marsh	Leaves	Suc	6	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	11.4		
			Fru	13.4		
	Saline lake		Suc	~10		Albert and Popp 1978
			Glu	~10		
			Fru	~10		
Suaeda maritima subsp. *pannonica* (Beck) Soó ex P.W.Ball [= *Suaeda pannonica* Beck]	Saline lake	Leaves	Suc	~5	mol m^{-3} PW	Albert and Popp 1978
			Glu	~15		
			Fru	~10		
Suaeda maritima subsp. *salsa* (L.) Soó [= *Suaeda salsa* (L.) Pall.]	Semi-arid salt-alkalinized grassland	Shoots	Man	31.1	µmol g^{-1} DW	Yang *et al.* 2012
Apocynaceae: *Cynanchum chinense* R.Br.	Semi-arid salt-alkalinized grassland	Shoots	Man	35.8	µmol g^{-1} DW	Yang *et al.* 2012
Asteraceae: *Artemisia anethifolia* Weber ex Stechm.	Semi-arid salt-alkalinized grassland	Shoots	Man	27.3	µmol g^{-1} DW	Yang *et al.* 2012
Artemisia santonicum L. [= *Artemisia monogyna* Waldst. & Kit.]	Saline lake	Leaves	Suc	~15	mol m^{-3} PW	Albert and Popp 1978
			Glu	~15		
			Fru	~30		
			Ino (Myo-i)	5		
Artemisia scoparia Waldst. & Kit.	Semi-arid salt-alkalinized grassland	Shoots	Man	47.6	µmol g^{-1} DW	Yang *et al.* 2012

Inula japonica Thunb.	Semi-arid salt-alkalinized grassland	Shoots	Man	37.3	µmol g^{-1} DW	Yang *et al.* 2012
Kalimeris integrifolia Turcz. ex DC.	Semi-arid salt-alkalinized grassland	Shoots	Man	33.9	µmol g^{-1} DW	Yang *et al.* 2012
Sonchus arvensis L.	Saline lake	Leaves	Suc	~20	mol m^{-3} PW	Albert and Popp 1978
			Glu	~15		
			Fru	~15		
			Ino (Myo-i)	6.4		
	Semi-arid salt-alkalinized grassland	Shoots	Man	64.8	µmol g^{-1} DW	Yang *et al.* 2012
Tripolium pannonicum (Jacq.) Dobrocz. [= *Aster tripolium* L.]	Salt marsh	Leaves	Suc	40	µmol g^{-1} DW	Briens and Larher 1982
			Glu	11		
			Fru	16		
		Roots	Suc	115		
			Glu	12		
			Fru	24		
		Leaves	Suc	2.4	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	1.4		
			Fru	4.6		
			Ino	0.6		
		Florets	Suc	4.9		
			Glu	17.1		
			Fru	41.8		
			Ino	3.2		
	Saline lake	Leaves	Suc	~35		Albert and Popp 1978
			Glu	~10		
			Fru	~25		
			Ino (Myo-i)	5.3		

Boraginaceae: *Tournefortia sibirica* L. var. *sibirica* [= *Messerschmidia sibirica* (L.) L.]	Semi-arid salt-alkalinized grassland	Shoots	Man	41.3	µmol g^{-1} DW	Yang *et al.* 2012
Brassicaceae: *Lepidium cartilagineum* (J. Mayer) Thell. [= *Lepidium crassifolium* Waldst. & Kit.]	Saline lake	Leaves	Suc	~5	mol m^{-3} PW	Albert and Popp 1978
			Glu	~15		
			Fru	~15		
			Ino (Myo-i)	8.5		
Caryophyllaceae: *Spergularia media* (L.) C.Presl.	Salt marsh	Leaves	Suc	6.9	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	20.6		
			Fru	18		
			Pin	32.3		
	Saline lake		Suc	~15		Albert and Popp 1978
			Glu	~8		
			Fru	~10		
			Ino (Myo-i)	2		
			Pin	33.5		
Combretaceae: *Lumnitzera littorea* (Jack) Voigt	Mangrove	Leaves	Man	112	mol m^{-3} PW	Popp *et al.* 1985
Lumnitzera racemosa Willd.	Mangrove	Leaves	Suc	5.9	mol m^{-3} PW	Popp 1984
			Glu	7.5		
			Fru	7.2		
			Ino (Myo-i)	1		
			Man	100		
Euphorbiaceae: *Excoecaria agallocha* L.	Mangrove	Leaves	Suc	15.7	mol m^{-3} PW	Popp 1984
			Glu	29		
			Fru	34.2		
			Ino (Myo+Chiro-i)	7.7		
			Que	88.5		
Leguminosae: *Astragalus complanatus* Bunge	Semi-arid salt-alkalinized grassland	Shoots	Man	56.9	µmol g^{-1} DW	Yang *et al.* 2012

Lespedeza juncea (L.f.) Pers. [= *Lespedeza hedysaroides* (Pall.) Kitag.]	Semi-arid salt-alkalinized grassland	Shoots	Man	51.2	µmol g^{-1} DW	Yang *et al.* 2012
Melilotus officinalis (L.) Pall.	Semi-arid salt-alkalinized grassland	Shoots	Man	66	µmol g^{-1} DW	Yang *et al.* 2012
Lythraceae: *Sonneratia alba* Sm.	Mangrove	Leaves	Suc	10.1	mol m^{-3} PW	Popp 1984
			Glu	21.7		
			Fru	25.4		
			Ino (Myo-i)	1.7		
			Man	200		
			Pin	1.8		
Malvaceae: *Commersonia fraseri* J.Gay	Mangrove	Leaves	Suc	22.2	mol m^{-3} PW	Popp 1984
			Glu	12.2		
			Fru	21.9		
			Ino (Myo+Scy-i)	19.7		
Heritiera littoralis Aiton	Mangrove	Leaves	Suc	33	mol m^{-3} PW	Popp 1984
			Glu	23.4		
			Fru	25.9		
			Ino (Myo-i)	0.6		
			Pin	1.9		
Hibiscus tiliaceus L.	Mangrove	Leaves	Suc	~20	mol m^{-3} PW	Popp 1984
Meliaceae: *Melia azedarach* L.	Mangrove	Leaves	Suc	119.8	mol m^{-3} PW	Popp 1984
			Glu	75.2		
			Fru	84.8		
			Ino (Myo-i)	32.7		
Xylocarpus granatum J. Koenig	Mangrove	Leaves	Suc	~100	mol m^{-3} PW	Popp 1984
			Glu	~100		
			Fru	~90		
			Ino (Myo+Chiro-i)	~41.9		
Xylocarpus mekongensis Pierre	Mangrove	Leaves	Suc	32.8	mol m^{-3} PW	Popp 1984

			Glu	8.4		
			Fru	7.7		
			Ino (Myo+Chiro-i)	7.6		
Myrtaceae: *Melaleuca hypericifolia* Sm.	Mangrove	Leaves	Suc	17.6	mol m^{-3} PW	Popp 1984
			Glu	13		
			Fru	17.7		
			Ino (Myo-i)	15.8		
			Que	4.4		
Osbornia octodonta F.Muell.	Mangrove	Leaves	Suc	51.3	mol m^{-3} PW	Popp 1984
			Glu	40.4		
			Fru	81.2		
			Ino (Myo+Scy-i)	3.1		
			Pin	5.5		
Picrodendraceae: *Micrantheum hexandrum* Hook.f.	Mangrove	Leaves	Suc	62.6	mol m^{-3} PW	Popp 1984
			Glu	18.4		
			Fru	19.1		
			Ino (Myo-i)	26.2		
Plantaginaceae: *Plantago maritima* L.	Salt marsh	Leaves	Suc	82	µmol g^{-1} DW	Briens and Larher 1982
			Glu	93		
			Fru	21		
		Roots	Suc	133		
			Glu	57		
			Fru	21		
	Saline lake	Leaves	Suc	~4	mol m^{-3} PW	Albert and Popp 1978
			Glu	~5		
			Fru	~2		
Plumbaginaceae: *Aegialitis annulata* R.Br.	Mangrove	Leaves	Suc	~60	mol m^{-3} PW	Popp 1984
			Ino (Chiro-i)	~80		
			Pin	~55		
				53		Popp and Polania 1989

		Twigs		30		
Limonium vulgare Mill.	Salt marsh	Leaves	Suc	76	µmol g^{-1} DW	Briens and Larher 1982
			Glu	14		
			Fru	14		
		Roots	Suc	966		
			Glu	117		
			Fru	155		
Primulaceae: *Aegiceras corniculatum* (L.) Blanco	Mangrove	Leaves	Man	~250	mol m^{-3} PW	Popp 1984
				248		Popp and Polania 1989
		Twigs		175		
		Leaves		287		Popp *et al.* 1985
Lysimachia maritima (L.) Galasso, Banfi & Soldano [= *Glaux maritima* L.]	Salt marsh	Leaves	Suc	12	mol m^{-3} PW	Gorham *et al.* 1980
			Glu	1.6		
			Fru	1.9		
			Ino	9.6		
Rhizophoraceae: *Bruguiera exaristata* Ding Hou	Mangrove	Leaves	Pin	~150	mol m^{-3} PW	Popp 1984
Bruguiera gymnorhiza (L.) Lam.	Mangrove	Leaves	Pin	~100	mol m^{-3} PW	Popp 1984
Ceriops tagal (Perr.) C.B.Rob.	Mangrove	Leaves	Suc	22.2	mol m^{-3} PW	Popp 1984
			Glu	8.8		
			Fru	10		
			Ino (Myo-i)	2.3		
			Pin	182		
Rhizophora apiculata Blume	Mangrove	Leaves	Pin	~220	mol m^{-3} PW	Popp 1984
Rhizophora x *lamarckii* Montr.	Mangrove	Leaves	Pin	~195	mol m^{-3} PW	Popp 1984
Rhizophora stylosa Griff.	Mangrove	Leaves	Pin	~175	mol m^{-3} PW	Popp 1984
		Twigs	Ino (Muco-i)	186		Popp and Polania 1989
		Roots		283		

Rubiaceae: *Opercularia volubilis* R.Br. ex Benth.	Mangrove	Leaves	Suc	5.6	mol m^{-3} PW	Popp 1984
			Glu	25.3		
			Fru	11.8		
			Ino (Myo-i)	3.2		
Scyphiphora hydrophylacea C.F.Gaertn.	Mangrove	Leaves	Suc	5.4	mol m^{-3} PW	Popp 1984
			Glu	91.3		
			Fru	6.8		
			Ino (Myo-i)	1.2		
			Man	~240		

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Table 1. Examples of relative increases of soluble carbohydrates in halophytes growing under controlled saline treatments.

Concentration of carbohydrates were expressed as increases (-fold) calculated from data obtained in tables or graphs. Abbreviations; Suc, sucrose; Glu, glucose; Fru, fructose; Ino, inositol – Chiro-i, *chiro*-inositol; Myo-i, *myo*-inositol –; Man, mannitol; Pin, pinitol; Sor, sorbitol; Red, reducing carbohydrates; Sol, total soluble carbohydrates.

Species	Plant material	Experimental conditions	CHO increases (-fold)	References
Monocotyledoneae				
Juncaceae: *Juncus maritimus* Lam.	2 to 5-month-old plants (leaves)	Hydroponic. NaCl treatment 0-300 mM 3 weeks	Suc (1.2), Glu (3.7), Fru (3.4)	Gorham *et al.* 1981
Juncaginaceae: *Triglochin maritima* L.	Adult plants (shoots, roots)	Treatments 0-100% seawater 2 weeks	Red (1.4, 4)	Jefferies *et al.* 1979
Poaceae: *Paspalum vaginatum* Sw.	Adult plants (shoots)	NaCl treatment 0-49.7 dS m^{-1}	Fru (2.7), Myo-i (2)	Lee *et al.* 2008
Phragmites australis (Cav.) Trin. ex Steud.	Juvenile plants cultured for 21 days (rhizomes)	Hydroponic. NaCl treatment 1.5-10‰ NaCl 16 days	Sol (3.5)	Hartzendorf and Rolletschek 2001
Dicotyledoneae				
Aizoaceae: *Mesembryanthemum crystallinum* L.	8.5-week-old plants (leaves)	500 mM NaCl treatments by daily irrigation for 5 days	Pin (4.7)	Vernon and Bohnert 1992a
Sesuvium portulacastrum (L.) L.	Explants cultured > 1 year (axillary shoots)	NaCl treatment 0-200 mM 30 days	Sol (1.9)	Lokhande *et al.* 2011
Amaranthaceae: *Atriplex halimus* L.	40-day-old plants (leaves)	NaCl treatment 0-550 mM 30 days	Suc (5)	Alla *et al.* 2012
Atriplex portulacoides L. [= *Halimione portulacoides* (L.) Aellen]	Adult plants (roots)	Treatments 0-100% seawater 2 weeks	Red (58)	Jefferies *et al.* 1979
Chenopodium quinoa Willd.	3-week-old seedlings (roots, adult-young leaves and stems)	NaCl treatment 0-500 mM 4 weeks	Sol (~4.1, ~1.7-3.3, ~3.1-2.4)	Eisa *et al.* 2012
Salicornia rubra A. Nelson [= *Salicornia europaea* L. subsp. *rubra* (A.Nelson) Breitung]	60-day-old plants (succulent stems)	Hydroponic. NaCl treatment 10-100 mM 6 hours	Sol (4.9)	McNulty 1985
Suaeda glauca (Bunge) Bunge	4-month-old plants (leaves)	NaCl treatment 0-900 mM 7 days	Sol (~2)	Jia *et al.* 2011
Brassicaceae: *Thellungiella salsuginea* (Pallas) O.E.Schulz [= *Thellungiella*	4-week-old seedlings (leaves)	NaCl treatments 0-500 mM 3 weeks	Sol (4.7)	Inan *et al.* 2004

halophila (C.A.Mey.) O.E.Schulz]				
Caryophyllaceae: *Honckenya peploides* (L.) Ehrh.	3-month-old plants (leaves)	Hydroponic. NaCl treatment 0-250 mM 3 weeks	Suc (10), Pin (2.2)	Gorham *et al.* 1981
Asteraceae: *Tripolium pannonicum* Jacq. Dobrocz. subsp. *tripolium* (L.) Greuter [= *Aster tripolium* L.]	2-month-old plants (roots)	Hydroponic. NaCl treatment 0-500 mM 4 weeks	Sol (1.7)	Geissler *et al.* 2009
	6-month-old plants (leaves)	Hydroponic. 0-300 mM 3 weeks	Suc (3.1), Fru (3.9), Ino (2.9), Pin (1.4)	Gorham *et al.* 1981
	Adult plants (shoots)	Hydroponic. NaCl treatment 0-150 mM 16 days	Suc (1.8)	Matsumura *et al.* 1998
Plantaginaceae: *Plantago coronopus* L.	4-week-old seedlings (leaves, roots)	NaCl treatments 0-500 mM 2 weeks	Sor (65, 6.1)	Koyro 2006
Plantago crassifolia Forssk.	Adult plants (leaves)	NaCl treatments 0-500 mM 3 months	Sor (~1.6)	Vicente *et al.* 2004
Plantago maritima L.	5-week-old seedlings (shoots, roots)	Daily additions of 50 mM from 0-400 mM NaCl	Sor (~10, ~230)	Ahmad *et al.* 1979
Plumbaginaceae: *Limonium perezii* (Stapf) F.T. Hubb.	3-week-old seedlings (leaves)	NaCl treatment 2.5-30 dS m^{-1} 67 days	Glu (~2.3), Fru (~2.3), Chiro-i (8.3), Sol (~3.5)	Liu and Grieve 2009
Limonium vulgare Mill.	Adult plants (shoots, roots)	Treatments 0-100% seawater 2 weeks	Red (1.6, 4.7)	Jefferies *et al.* 1979
Rhizophoraceae: *Bruguiera parviflora* (Roxb.) Wight & Arn. ex Griff.	2-month-old plants (leaves)	Hydroponic. NaCl treatment 0-400 mM 45 days	Sol (2.5)	Parida *et al.* 2002
Kandelia candel (L.) Druce	3-month-old plants (leaves)	NaCl treatment 0-500 mM 45 days	Man (2.3), Pin (1.7), Sol (1.7)	Zhu *et al.* 2011