Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress

Inés Slama1, Chedly Abdelly1, Alain Bouchereau2, Tim Flowers3 and Arnould Savouré4,*

1Laboratoire des Plantes Extremophiles, Centre de Biotechnologie de Borj-Cedria (CBBC), BP 901, Hammam-Lif 2050, Tunisia, 2UMR 1349 IGEPP, INRA/Agrocampus Ouest/Université de Rennes 1, Domaine de la Motte, BP 35327, 35653 Le Rheu Cedex, France, 3School of Life Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QG, UK and 4Sorbonne Universités, UPMC Université Paris 06, Adaptation de Plantes aux Contraintes Environnementales, URFS, Case 156, 4 place Jussieu, F-75252 Paris cedex 05, France

* For correspondence. E-mail arnould.savoure@upmc.fr

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Background and Aims Osmolytes are low-molecular-weight organic solutes, a broad group that encompasses a variety of compounds such as amino acids, tertiary sulphonium and quaternary ammonium compounds, sugars and polyhydric alcohols. Osmolytes are accumulated in the cytoplasm of halophytic species in order to balance the osmotic potential of the Na\(^+\) and Cl\(^-\) accumulated in the vacuole. The advantages of the accumulation of osmolytes are that they keep the main physiological functions of the cell active, the induction of their biosynthesis is controlled by environmental cues, and they can be synthesized at all developmental stages. In addition to their role in osmoregulation, osmolytes have crucial functions in protecting subcellular structures and in scavenging reactive oxygen species.

Scope This review discusses the diversity of osmolytes among halophytes and their distribution within taxonomic groups, the intrinsic and extrinsic factors that influence their accumulation, and their role in osmoregulation and osmoprotection. Increasing the osmolyte content in plants is an interesting strategy to improve the growth and yield of crops upon exposure to salinity. Examples of transgenic plants as well as exogenous applications of some osmolytes are also discussed. Finally, the potential use of osmolytes in protein stabilization and solvation in biotechnology, including the pharmaceutical industry and medicine, are considered.

Key words: Abiotic stress, genetic engineering, halophytes, osmolytes, osmoregulation, osmoprotection, osmotic adjustment, salinity stress, salt tolerance.

INTRODUCTION

Environmental stress (an environmental perturbation causing a response in the plant; Cheeseman, 2013) factors such as drought, salinity, chilling, freezing and high temperatures affect plant growth and generate a threat to sustainable agriculture. In field conditions, crops and other plants are routinely subjected to a combination of different abiotic stresses (Boyer, 1982; Ahuja et al., 2010; Atkinson and Urwin, 2012). For example, heat stress is often accompanied by water deficiency and drought by salinity. This has become an important issue due to concerns about the effects of climate change on plant resources, biodiversity and global food scarcity (with almost 70 papers published between 2010 and early 2013 containing the words ‘food’, ‘climate’ and ‘change’ in their title). Soil salinization is a serious soil degradation, which can arise from natural causes and human-mediated activity such as forest clearance or irrigation with low-quality water (Pitman and Lauchaüli, 2004), especially in regions with high rates of evapotranspiration. Salt-affected soils are found in more than 100 countries of the world, especially those in arid and semi-arid zones, with a variety of extents, types and properties (Rengasamy, 2006). Approximately 20% of the irrigated lands in the world suffer from salinization and it has been estimated that the world as a whole has been losing at least 0.5–1% of irrigated area every year (Munns and Tester, 2008).

Under saline conditions, salt-sensitive glycophytes (plants of sweet or fresh water and which do not qualify as halophytes), a group of plants to which the majority of crops belong, have to cope with osmotic and ionic stresses. The former affects plants as a rise in salt levels around the roots leads to inhibition of water uptake, cell expansion and lateral root development (Munns and Tester, 2008). The latter develops when ions such as Na\(^+\) and Cl\(^-\) accumulate in excess in plants, particularly in leaves, leading to a decrease in the activity of primary metabolism, including photosynthesis, with an increase in chlorosis and cell death (Glenn et al., 1999).

Physiological and molecular genetic studies have led to increasing knowledge of the protective mechanisms that plants use to cope with the detrimental effects of salinity (Zhu, 2002; Munns and Tester, 2008; Horie et al., 2009). During salt stress, Na\(^+\) and Cl\(^-\) enter cells and their over-accumulation can modify the lipid and protein composition of plant cell plasma membranes as well as the function and regulation of important membrane proteins, such as water channels and Na\(^+\).
transporters (Horie and Schroeder, 2004; Ward et al., 2009) and signalling molecules (Zhu, 2002). Halophytes, extremophiles found on salt marshes, in saline depressions (sebkhas), in saline inland deserts and on sand dunes or rocky coasts, have evolved a number of adaptive traits expressed at various levels of organization that allow them to germinate, grow and achieve their complete cycle of development under such conditions (Flowers and Colmer, 2008). Foremost amongst these is the control of ion concentrations in the plants and their cells.

Osmoregulation and/or osmoprotection and ion homeostasis through compartmentalization are part of the main adaptive mechanisms found in halophytes subjected to salinity (Flowers and Colmer, 2008). Osmotic adjustment in halophytes is achieved through the accumulation of energetically cheap inorganic ions, such as Na⁺ and Cl⁻, and low-molecular-weight organic solutes, collectively known as osmolytes (Yeo, 1983; Glenn et al., 1999). The ions are primarily located in the vacuoles and the osmolytes (amino acids, tertiary sulphonium compounds, quaternary ammonium compounds, sugars and polyhydric alcohols) in the cytoplasm. In addition, osmolytes protect macromolecular sub-cellular structures and mitigate oxidative damage caused by free radicals produced in response to these abiotic stresses. Elevated levels of osmolyte accumulation in plant cells have been correlated with enhanced stress tolerance through the scavenging of free radicals and protecting enzymes (Szabados et al., 2011).

This review discusses the diversity of osmolyte accumulation in halophytes, the intrinsic and extrinsic factors that influence their accumulation and their roles in osmoregulation and osmoprotection. The high structural diversity and metabolic origins of osmolytes combined with their multifunctionality and the seasonal flexibility of metabolism in plants facing multiple stresses are also discussed. Since the engineering of osmolyte metabolism can lead to opportunities to improve plant tolerance of environmental stresses, examples of transgenic plants engineered to have enhanced osmolyte content as well as the effects of exogenous applications of some osmolytes will also be described.

**CONTRIBUTION OF SODIUM TO OSMOTIC ADJUSTMENT IN HALOPHYTES**

External sodium chloride can impose on plants an initial water deficit, injury to plasma membranes, accumulation of reactive oxygen species (ROS), impairment of photosynthesis and changes in respiration and nitrogen assimilation (Munns and Tester, 2008). In addition, by effectively replacing K⁺ (a cofactor in a number of enzymatic processes) in the cytoplasm with Na⁺, critical biochemical processes such as protein synthesis are inhibited. Halophytes have evolved mechanisms for selective Na⁺ and Cl⁻ sequestration into the vacuole, allowing the cytoplasm to be maintained at substantially lower concentrations of these ions, avoiding their inhibitory effects on metabolic processes (Flowers et al., 1986; Flowers and Colmer, 2008; Flowers et al., 2014).

Accumulation of Na⁺, often associated with that of Cl⁻, is prominent in shoots of most halophytic dicotyledonous plants, particularly those of the Amaranthaceae (Chenopodiaceae; Flowers, 1985; Albert et al., 2000). Chenopodiaceous halophytes growing in 100–200 mM NaCl can contain some 500 mM Na⁺ and 340 mM Cl⁻ (Flowers, 1985), concentrations that contribute significantly to the internal osmotic potential and to turgor generation [although the latter may be compromised by salt in the cell walls (Clipson et al., 1985; Flowers et al., 2014)]. When freely available in the soil solution, Na⁺ and Cl⁻ constitute readily available solutes provided that, at the cell level, they are efficiently sequestered in vacuoles. Such compartmentalization is hypothesized to avoid their damaging effects on the cytosol and its organelles. This model of the cellular basis of salt tolerance implies the ability to maintain a sufficient uptake of K⁺ in order to maintain a high cytosolic K⁺/Na⁺ ratio, one of the key determinants of plant salt tolerance.

In spite of the wide availability of Na⁺ and Cl⁻, their contribution to osmotic adjustment in halophytic species varies among families and appears to be related to the concentrations of accumulated organic solutes (Flowers et al., 1977; Flowers and Colmer, 2008).

**WHAT IS AN OSMOLYTE?**

One of the metabolic consequences of osmotic stress is the accumulation of osmolytes, low-molecular-weight organic compounds, also known as compatible solutes, that are highly soluble and do not interfere with normal metabolic reactions because they are non-toxic even at high cellular concentrations (Flowers et al., 1977; Wyn Jones et al., 1977; Yancey, 2005). Osmotic adjustment has traditionally been accepted to be the primary function of osmolytes in plants (Hasegawa et al., 2000), but this is not always the case, especially in glycophytes exposed to salt. For example, proline accumulation in Arabidopsis thaliana is too low to play a role in the osmotic adjustment of the cells (Liu and Zhu, 1997; Ghars et al., 2008). In addition, the concentration (expressed on the basis of tissue mass or tissue water) of osmolytes can be significantly lower than that of inorganic solutes in some halophytes (Gagneul et al., 2007). These osmolytes presumably have other functions (Bartels and Sunkar, 2005; Hare et al., 1998; Szabados et al., 2011). Indeed some of these compounds can modify the solvent properties of water, stabilize the internal osmotic potential, increase the thermodynamic stability of folded proteins and protect macromolecular structures (Yancey, 2005). Because some of these solutes also protect cellular components from dehydration injury, they are commonly referred to as osmoprotectants. In halophytes several types of osmolyte can often be accumulated simultaneously, although the abundance of a particular osmolyte may depend on cell compartment, organ, developmental stage and environmental conditions (Murakeözy et al., 2003; Gagneul et al., 2007). This raises the question of their individual or combined (possibly synergistic) mode of action in protecting cells against the environment (Yancey, 2005). Accumulation processes that determine the mode of action of osmolytes are the result of the coordinated regulation of biosynthetic and catabolic pathways. Among osmolytes, some are subject to rapid and short-term fluctuations (accumulation/degradation, e.g. proline), while others accumulate for longer periods (e.g. betaines) (Gagneul et al., 2007). Metabolic dynamics also has adaptive significance to the extent that the metabolic pathway involved in the production or consumption
of a compatible osmolyte can be as important as the terminal product itself. For example, the biosynthesis and catabolism of proline contributes to rapid and efficient consumption or release of reducing power (Szabados and Savouré, 2010).

Not only the biochemical/metabolic nature of the osmolyte but also the seasonal pattern of osmolyte accumulation show significant species-specific fluctuations. In addition, the cellular levels of osmolytes can change according to growth period, developmental stage, organ and environmental parameters (Hare et al., 1998; Murakéozy et al., 2003). Sub-cellular partitioning of osmolytes between cell compartments has also been demonstrated to be modulated according to growing conditions, nutritional and environmental challenges (Aubert et al., 2009, Gagneul, 2007).

DIVERSITY OF OSMOLYTE COMPOUNDS IN THE PLANT KINGDOM

A wide range of osmoprotective compounds has been identified, including mono-, di-, oligo- and polysaccharides, such as glucose, fructose, sucrose, trehalose, raffinose and fructans; sugar alcohols (polyols) such as sorbitol, mannitol, glycerol, inositol and methylated inositolis; amino acids, such as proline, piperolic acid; methylated proline-related compounds, such as methyl-proline, proline betaine and hydroxyproline betaine; other betaines, such as glycine betaine, β-alanine betaine, choline O-sulphate; and tertiary sulphonium compounds, such as dimethylsulphoniopropionate (DMSP) (Rhodes et al., 2002; Ashraf and Foolad, 2007) (Fig. 1). In the following sections, the most common osmolytes in halophytes are described in detail.

**Amino acids**

It has been reported that amino acids, such as proline, alanine, arginine, glycine, amides such as glutamine and asparagine, and the non-protein amino acids γ-aminobutyric acid, piperolic acid, citrulline and ornithine are accumulated in higher plants under conditions of abiotic stress (Ahmad et al., 1981; Mansour, 2000). These compounds are synthesized from the primary metabolic pathways that lead to metabolically inactive (safe) molecules (Rhodes et al., 2002).

Proline is known to occur widely in higher plants and can be accumulated in considerable amounts in response to salt stress and drought (Kavi Kishor et al., 2005). In plants, proline is synthesized mainly from glutamate, which is reduced to glutamate semialdehyde by pyrroline-5-carboxylate synthetase (P5CS) (a key enzyme in proline biosynthesis) and spontaneously converted to pyrroline-5-carboxylate (P5C) (Szabados and Savouré, 2010). P5C reductase (P5CR) further reduces the P5C intermediate to proline. Catabolism of proline occurs in mitochondria via the sequential action of proline dehydrogenase or proline oxidase (ProDH), producing P5C from proline, and P5C dehydrogenase (P5CDH), which converts P5C to glutamate (Szabados and Savouré, 2010; Servet et al., 2012).

In addition to glutamate, the other precursor for proline biosynthesis is ornithine, which is transaminated to P5C by a mitochondrial ornithine-δ-aminotransferase (Armengaud et al., 2004; Verbruggen and Hermans, 2008). Proline is also involved in the alleviation of cytoplasmic acidosis and sustaining NADP+/NADPH ratios at the levels required for metabolism (Hare and Cress, 1997). Proline accumulated under stress conditions might therefore serve as a sink for excess reductants, providing the NAD⁺ and NADP⁺ necessary for maintenance of respiratory and photosynthetic processes. High

![Fig. 1. Structures of common osmolytes identified in halophytes.](https://academic.oup.com/aob/article-abstract/115/3/433/304998/Diversity-distribution-and-roles-of-osmoprotective)
concentrations of NADP⁺ are necessary for the pentose phosphate pathway for the regeneration of NADPH and to supply ribose-5-phosphate for the synthesis of purines (Kavi Kishor et al., 2005).

Quaternary ammonium compounds

The quaternary ammonium compounds accumulated in plants are glycine betaine, \( \beta \)-alanine betaine, proline betaine, choline-O-sulphate, hydroxyproline betaine and pipoccolate betaine (Ashraf and Harris, 2004).

Glycine betaine is accumulated by numerous organisms, including bacteria, cyanobacteria, algae, fungi and animals (Türkan and Demiral, 2009). Among the variety of quaternary ammonium compounds, glycine betaine is one of the most abundantly occurring in plants exposed to dehydration due to salinity, drought, heat and cold stress (Hanson et al., 1991; Guo et al., 2009; Lokhande and Suprasanna, 2012). Glycine betaine is synthesized mainly from choline, which is converted to betaine aldehyde and then to glycine betaine through the sequential enzymatic action of choline monoxygenase (CMO) and betaine aldehyde dehydrogenase (BADH), respectively. Although other pathways, such as the direct \( \text{N}^-\text{methylolation of glycine, are also known, the choline pathway to glycine betaine has been identified in all glycine betaine-accumulating plant species (Ashraf and Foolad, 2007; Fitzgerald et al., 2009).

\( \beta \)-Alanine betaine is synthesized by the \( S \)-adenosylmethionine-dependent \( N \)-methylolation of \( \beta \)-alanine via \( N \)-methyl \( \beta \)-alanine and \( N,N \)-dimethyl \( \beta \)-alanine. In Limonium species, purified \( N \)-methyltransferase (NMTase) is trifunctional, methylating \( \beta \)-alanine, \( N \)-methyl \( \beta \)-alanine and \( N,N \)-dimethyl \( \beta \)-alanine (Rathinasabapathi et al., 2001). Nevertheless, the metabolic origin of \( \beta \)-alanine in \( \beta \)-alanine betaine-producing species remains speculative since multiple routes have been proposed deriving from aliphatic polyamines, propionate or uracil. These pathways may cooperate for \( \beta \)-alanine and \( \beta \)-alanine betaine production in Limonium latifolium (Duhaze et al., 2003). It has been proposed by Hanson et al. (1991) that \( \beta \)-alanine betaine is a more suitable osmoprotectant than glycine betaine under saline hypoxic conditions because the first step in glycine betaine synthesis requires molecular oxygen. Furthermore, \( \beta \)-alanine betaine accumulation was proposed to be an evolutionary strategy to avoid metabolic competition for choline (Hanson et al., 1994), because \( \beta \)-alanine betaine is synthesized from the ubiquitous primary metabolite \( \beta \)-alanine (Duhaze et al., 2003).

Proline betaine, a dimethyl proline also called stachydrine, which accumulates in non-halophytic Citrus and Medicago species (Nolte et al., 1997; Trinchant et al., 2004), has a much more scattered distribution than glycine betaine in halophytes. Methylated proline has been shown to accumulate in a few halophytic species of the Plumbaginaceae, Capparidaceae, Myrtaceae, Rutaceae, Labiatae, Compositae and Leguminosae (Wyn Jones and Storey, 1981; Rhodes and Hanson, 1993; Hanson et al., 1994; Naidu, 2003; Carter et al., 2005). In bacteria, the osmoprotective role of proline betaine has been clearly established, proline betaine being a more effective osmoprotectant than proline (Hanson et al., 1994). Partial investigations in plants indicate that proline betaine accumulation represents a long-term response to salinization deriving from several methylation steps of proline (Trinchant et al., 2004).

Tertiary sulphonium compounds

Tertiary sulphonium compounds such as DMSP contain a fully methyl substituted sulphur atom (Larher et al., 1977). Dimethylsulphoniopropionate is synthesized in many algae, in contrast to plants, where this compound is only found in a few salt marsh grasses of the genus Spartina, in sugar canes (Saccharum spp.) and in Wollastonia biflora (Otte et al., 2004). Plants and algae that produce DMSP synthesize this compound from methionine, but the pathways from methionine to DMSP differ between plant groups and species (Hanson et al., 1994; Koecis and Hanson, 2000). In algae, methionine is first transaminated to form 4-methylthio-2-oxobutyrate, but in higher plants it is first methylated to form S-methyl methionine, which is then converted directly to dimethylsulphoniopropionaldehyde (DMSP-ald) without formation of dimethylsulphonipropylamine, most likely via a transamination/decarboxylation mechanism. Interestingly, grasses such as Spartina alterniflora have evolved specific enzymes that mediate the conversion of S-methyl methionine to DMSP-ald (Koecis and Hanson, 2000). Although many functions of DMSP have been suggested, such as a detoxifier of excess sulphur, an antioxidant and a herbivore deterrent, the possible involvement of DMSP in osmoregulation has received by far the most attention. This is partly due to the structural similarity of this tertiary sulphur compound to quaternary ammonium compounds such as glycine betaine. In S. alterniflora, DMSP is produced in high concentration in green tissues, where it can represent up to 86% of the total concentration of sulphur, but independently of the external salinity. Dimethylsulphoniopropionate could be involved in osmoregulation without changes in its concentrations at the tissue level by movement between the cytoplasm and the vacuoles within the cells, depending on the osmotic potential of the cytoplasm (Otte et al., 2004).

Sugars

Sugars such as sucrose and trehalose have been shown to be accumulated in plants in response to abiotic stresses (Briens and Larher, 1982; Yuanyuan et al., 2009). Besides their possible roles in osmotic adjustment and in stabilizing membranes upon water stress (Lokhande and Suprasanna, 2012), these compounds may play several other important regulatory functions in stressed plants. Sugars not only sustain the growth of sink tissues, but also affect sugar-sensing systems that regulate the expression, either positively or negatively, of a variety of genes involved in photosynthesis, respiration and the synthesis and degradation of starch and sucrose (Hare et al., 1998). Trehalose, a non-reducing disaccharide, is highly soluble but chemically unreactive, making it compatible with cellular metabolism even at high concentrations. Trehalose is present in significant concentrations in several bacteria and fungi but rare in vascular plants (Lunn et al., 2014, Fernandez et al., 2010). In plants, the accumulation of trehalose was first demonstrated in resurrection plants upon desiccation, but remains, to our knowledge, to be demonstrated in halophytes.
Sugar alcohols

In general, sugar alcohols are divided between acyclic (e.g. mannitol) and cyclic (e.g. pinitol) polyols. The accumulation of polyols may have dual functions: facilitating osmotic adjustment and supporting redox control. Mannitol, which is not widely accumulated in halophytes, is synthesized from fructose-6-phosphate by subsequent enzymatic action of mannose-6-phosphate isomerase (phosphomannose isomerase), mannose-6-phosphate reductase and mannose-1-phosphate phosphatase (Loescher et al., 1992). Although the function of this osmolyte has not been well investigated in halophytes, a possible role in Prosopis strombulifera is stabilizing macromolecular structures and promoting scavenging systems for reactive oxygen species (Llanes et al., 2013).

The most frequently reported form of cyclic polyol in halophytes is pinitol, which is derived from the methylation of myo-inositol and epimerization of ononitol by the actions of inositol-O-methyltransferase and ononitol epimerase (Sengupta et al., 2008). Myo-inositol is the most common of six isomers of inositol that occur in plant tissues and methylated derivatives of all these isomers have been isolated, with presumed important functions as protective and signalling compounds (Sureshan et al., 2009). The co-occurrence in Limonium species of chiro-inositol and pinitol raises questions about an alternative way of synthesizing pinitol in which epimerization from myo-inositol may precede the methylation step (Gagneul et al., 2007).

DISTRIBUTION OF OSMOLYTES AMONG HALOPHYTES

The diversity and threshold level of metabolites in plants can vary considerably according to species and to environmental conditions (Sanchez et al., 2008; Lugan et al., 2010; Szabados et al., 2011). Although increased accumulation of osmolytes by plants exposed to abiotic stresses has been reported, as mentioned above, not all plant species synthesize all kinds of osmolyte; some species synthesize and accumulate very low quantities of these compounds while some plant species not do so at all (e.g. Ashraf and Foolad, 2007). In general, osmolyte accumulation is reported in species that are continuously exposed to abiotic stresses; the synthesis of these osmolytes is an energy-dependent process that consumes large numbers of ATP molecules (Larher et al. 1977; Rhodes et al., 2002; Flowers and Colmer, 2008).

Halophytes of the three groups listed above (in the section What is an osmolyte?), usually accumulate one dominant osmolyte, which can be proline, glycine betaine, sorbitol, β-alanine betaine, choline-O-sulphate or sugar (Tipirdamaz et al., 2006; Arbona et al., 2010; Lugan et al., 2010). However, some halophytes accumulate more than one compatible solute (Gagneul et al., 2007). Based on their glycine betaine and proline accumulation potential, Tipirdamaz et al. (2006) categorized the halophytes from an inland salt marsh in Turkey. Their studies showed that the species that behaved as glycine betaine accumulators appeared to be poor proline accumulators and vice versa, although the correlation between the concentrations of the two solutes was not significant (our analysis). The level of glycine betaine accumulation reported in the halophytes analysed by Tipirdamaz et al. (2006) was 1.5–400 μmol g⁻¹ dry weight and some of the highest glycine betaine-accumulating halophytes were members of the Amaranthaceae (Chenopodiaceae): on average, the concentration in the chenopods (21 analyses) was 3.3-fold that in the other species (18) analysed. Osmolyte contents in representative families of halophytes are described in the following sections and in Table 1; more detail for individual species can be found in Supplementary Data Table S1.

Amaranthaceae

The Amaranthaceae (which include the former Chenopodiaceae) is a relatively large family, having about 174 genera and 2050 species, and represents the most species-rich lineage within the Caryophyllales. The largest number of halophytic species is found in this family. All the species are reported to accumulate glycine betaine, with the exceptions of C. quinoa (Tables 1 and Supplementary Data Table S1, and Yokoshi and Tanimoto, 1994; Ruffino et al., 2010) and Noaea mucronata (Tipirdamaz et al., 2006). It is clear that, where salinities are above the optimal for growth, the concentrations of proline and sugars increase, e.g. in Atriplex halimus (Bajji et al., 1998); Kochia sieversiana (Yang et al., 2007); Suaeda fruticosa (Hameed et al., 2012). The low glycine betaine and high proline concentrations reported for Atriplex hastata (Tipirdamaz et al., 2006) probably reflect that this species grows under such conditions. In a relatively early report, Guy et al. (1984) noted that, where glycine betaine was measured in shoots of Salicornia europaea grown at different salinities, the concentration of glycine betaine appeared to fall when expressed on a dry weight basis but plateaued when expressed on the basis of organic dry matter, highlighting the importance of the basis of expression of concentrations.

In addition the glycine betaine content may vary according to the type of photosynthesis, e.g. C3 type, dual-celled (Kranz) C4 type and the single-celled C4 type discovered in Amaranthaceae (Voznesenskaya et al., 2001). A general trend, pointed out by Larher et al. (2009), is that glycine betaine contents decreased from C3 plants to single-cell C4 plants, the C4 plants exhibiting Kranz anatomy being at an intermediate position. This observation suggests that different concentrations of glycine betaine might be needed to achieve osmoprotection according to the type of photosynthesis. However, these preliminary observations on glycine betaine content could also reflect variability in the level of glycine betaine according to the genus or the species selected for the study of Larher et al. (2009).

Plumbaginaceae

The Plumbaginaceae consist of 15 genera and 500–700 species. The family as a whole is tolerant of saline or dry conditions, although there is some specificity in the types of environmental cues (habitats) to which different groups in the family are adapted (Labbe, 1962). The biochemical diversity of organic osmolytes accumulated by Plumbaginaceae is rarely met in other halophytes (Larher and Hamelin, 1975; Rhodes and Hanson, 1993; Gagneul et al., 2007). Such metabolic diversity has been used as an example to explore the selective
advantages that the different solutes could have depending on the nature of the constraints and habitats (Hanson et al., 1994; Gagneul et al., 2007). Hanson et al. (1994) claimed that glycine betaine was dominant in species of the Plumbaginaceae adapted to dry environments and that this compound is not co-accumulated with ω-alanine betaine in these species. Although multiple quaternary ammonium compounds are found (Table 1), most members of this family accumulate choline-Ο-sulphate and ω-alanine betaine as osmoprotective compounds (Larher and Gagneul, 2001; Gagneul et al., 2007). Specifically, choline-Ο-sulphate can function in sulphate storage as well as in osmoprotection, ω-alanine betaine may be superior to glycine betaine in hypoxic saline (oxygen-depleted) conditions, and proline-derived betaines may be beneficial in chronically dry environments. Hanson et al. (1994) have shown that Aegialitis, a species of mangrove, accumulates choline-Ο-sulphate and have proposed that its biosynthesis serves to detoxify sulphate, a major anion in seawater that can be inhibitory at high concentrations (Hanson et al., 1994). A detailed metabolic study in L. latifolium under control and salt-stress conditions revealed that the contribution of betaines to osmotic potential remained low compared with sugars and cyclitols (Gagneul et al., 2007). The study of Gagneul et al. (2007) also indicated that long-term saline treatment did not induce (or suppress) the production of these solutes. Surprisingly, the uncommon betaines of L. latifolium were not found to be significantly osmoregulated (Gagneul et al., 2007).

While some families of halophytes produce high levels of nitrogenous compounds, others preferentially accumulate sugars and polyols. Sorbitol has been described as the most abundant soluble carbohydrate in all Plantago species, including taxa with different degrees of salt tolerance (Ahmad et al., 1979; Königshofer, 1983; Koyro, 2006; Pommernberg et al., 2007). In addition, rhamnose was found to be abundant in Plantago maritima roots (Briens and Larher, 1982).

From the study of Nadwodnik and Lohaus (2007), among Plantago species only sea plantain has a higher sorbitol concentration in the vacuoles of its mesophyll cells (by about 7-fold) than in the cytosol. The high sorbitol concentration in vacuoles of P. maritima indicates that sorbitol may have functions in this species additional to serving as a transport form of carbon, as postulated in non-halophytes. Contrasting results were obtained between P. maritima collected from inland salty areas surrounding a lake and those collected in coastal salt marshes (Ahmad et al., 1997; Tipirdamaz et al., 2006); surprisingly, proline and glycine betaine were found to accumulate in the lake group while these compounds were not detected in the salt-marsh plants. This discrepancy may be linked to plant metabolic adaptation to oxygen deficiency conditions as it occurs in marine salt marshes (Tipirdamaz et al., 2006). This needs to be further addressed.

### Aizoaceae

In the Aizoaceae, Lokhande et al. (2011) found a large increase in proline concentration in Sesuvium portulacastrum when callus and axillary shoot cultures were exposed to salt and drought alone or under iso-osmotic stress conditions of NaCl and polyethylene glycol (PEG). High proline accumulation has also been shown in S. portulacastrum plants exposed to various abiotic constraints, including salinity, drought and heavy metals (Messedi et al., 2004; Ghnaya et al., 2007; Slama et al., 2008). Furthermore, S. portulacastrum exposed to salinity exhibited increased synthesis of proline and polyalcohols such as myo-inositol, α- ononitol and α-pinitol (Ishitani et al., 1996; Lokhande et al., 2010). Salt induces the accumulation of methylated inositols, α-ononitol and α-pinitol in Mesembryanthemum crystallinum, as shown by the results of coordinate induction of several genes of the inositol synthesis pathway (Paul and Cockburn, 1989; Ishitani et al., 1996). Pinitol represented 71% of the soluble carbohydrate fraction and 9.7% of dry weight when plants were exposed to 400 mM NaCl (Paul and Cockburn, 1989). In contrast, Arabidopsis does not show upregulation of these genes or increases in these inositol-derived compounds in response to salt stress, which suggests an important role for these compounds in salt tolerance. Salt-stimulated levels of glycine betaine in S. portulacastrum have also been observed (Ramesh Kannan et al., 2013). Paul and Cockburn (1989) found that irrigation of M. crystallinum plants with 400 mM NaCl to induce crassulacean acid metabolism was accompanied by the accumulation of pinitol. Pinitol may function as a compatible solute in the cytosol and especially in the chloroplasts to counteract the presence of high concentrations of Na⁺ and Cl⁻ ions in the vacuoles. Such a pronounced accumulation of osmolytes and their physiological

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**Table 1. Osmolyte distribution in halophyte family among angiosperms**

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Osmolytes</th>
</tr>
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<tbody>
<tr>
<td>Monocotyledoneae</td>
<td>Poales</td>
<td>Sucrose, proline, low level of glycine betaine</td>
</tr>
<tr>
<td></td>
<td>Cyperaceae</td>
<td>Sucrose</td>
</tr>
<tr>
<td></td>
<td>Juncaceae</td>
<td>Proline, glycine, inositol</td>
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<td></td>
<td>Poaceae</td>
<td>Proline, piceolate, fructose, maltose, sucrose</td>
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<tr>
<td>Dicotyledoneae</td>
<td>Alismatales</td>
<td>Proline, glycine, sucrrose</td>
</tr>
<tr>
<td>Brassicales</td>
<td>Brassicaceae</td>
<td>Proline, sucrose, proline, myo-inositol, sucrose</td>
</tr>
<tr>
<td>Caryophyllales</td>
<td>Aizoaceae</td>
<td>Glycine betaine or proline, methylated onium compounds, sugars</td>
</tr>
<tr>
<td>Amaranthaceae</td>
<td>Plumbaginaceae</td>
<td>β-Alanine betaine, choline-Ο-sulphate, proline, piceolate, sucrose, glycine betaine in a few species</td>
</tr>
<tr>
<td>Fabales</td>
<td>Portulaceae</td>
<td>Proline</td>
</tr>
<tr>
<td>Lamiales</td>
<td>Casuarinaceae</td>
<td>Proline</td>
</tr>
<tr>
<td>Malpighiales</td>
<td>Acanthaceae</td>
<td>Glycine betaine</td>
</tr>
<tr>
<td>Myrtales</td>
<td>Rhizophoraceae</td>
<td>Proline, sucrose</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td>Combretaceae</td>
<td>Mannitol</td>
</tr>
<tr>
<td>Plantaginaceae</td>
<td>Plantaginaceae</td>
<td>Sorbitol, proline, rhamnose</td>
</tr>
<tr>
<td>Solanales</td>
<td>Fabaceae</td>
<td>Proline, glycine betaine, pinitol, sugars</td>
</tr>
<tr>
<td></td>
<td>Solaneaceae</td>
<td>glycine betaine</td>
</tr>
</tbody>
</table>
role in osmotic adjustment and anti-oxidative defence may have contributed to the success of these species in growing under adverse environmental stresses.

Poaceae

Many grasses accumulate glycine betaine (Supplementary Data Table S1), although this was not detected in **Puccinellia nuttalliana** (Guy et al., 1984). Soluble sugars are also commonly reported as components of the solute potential, which was the case for sucrose in **Agrostis stolonifera** and **Phragmites communis** growing in a sulphate-dominated soil in Austria: glycine betaine increased in the autumn in *A. stolonifera* (Hutterer and Albert, 1993). Proline, which is also commonly reported to make only a small contribution to the osmotic potential of **Distichlis spicata** (Fan et al., 1993; Marcum, 1999), appears at high NaCl concentrations in **Sporobolus virginicus** (Marcum and Murdoch, 1992). Although DMSP has been reported in species of *Spartina* (Supplementary Data Table S1), 3-DMSP was not detected in *Spartina townsendii* by Adrian-Romero et al. (1998). Salt induces the synthesis of pinotil in **Porteresia coarctata**, a halophytic wild rice, in contrast to its absence in domesticated rice (Sengupta et al., 2008). These authors (Sengupta et al., 2008) suggested that the enhanced synthesis of pinotil in *P. coarctata* under stress may be one of the adaptive features employed by the plant in addition to its known salt excretion mechanism.

Brassicaceae

Proline accumulation is widely observed in halophytes of the Brassicaceae (Table 1 and Supplementary Data Table S1) and salt-induced expression of proline biosynthetic genes have been reported frequently. For instance, the proline concentration increased in **Eutrema salsugineum** (Koch and German, 2013; formerly **Thellungiella salsuginea**, an arabidopsis relative and a model species for abiotic stress tolerance studies) under salt stress to reach a very high level (1600 µmol g⁻¹ dry weight) at 500 mM NaCl (Inan et al., 2004; Ghars et al., 2008). A comparative metabolomic approach between *Arabidopsis* and *Thellungiella* under salt-stress conditions revealed that *Thellungiella* appears to be able to cope with low water potentials by sustaining a water potential gradient with the environment, due to passive reduction in water content and constitutive over-concentration of osmolytes (Lugan et al., 2010). Megdiche et al. (2007) showed that the coastal halophyte **Cakile maritima** accumulates proline and soluble carbohydrates in its leaves during the period of intensive leaf growth. These organic compounds likely play a role in leaf osmotic adjustment and in the protection of membrane stability at severe salinity. A study reporting seasonal changes in the levels of compatible osmolytes in three halophytic species from inland saline vegetation in Hungary revealed that the high cytoplasmic concentration of proline observed in **Lepidium crassifolium** allows a decrease in osmotic potential of 50% (Murakézy et al., 2003). Although detected in several Brassicaceae species (Selvaraj et al., 1995; Xing and Rajashhekar 2001; Jdey et al., 2014), glycine betaine has never been found to play any osmotic role.

Other families

Increased accumulation of osmolytes is also observed in halophytes belonging to other families (Table 1), where accumulation of various sugars and polyols can also be involved in osmoregulatory mechanisms.

Conclusion

Osmolytes, including carbohydrates, quaternary ammonium compounds, proline and sulphonium compounds, are widely distributed among orders of flowering plants, reflecting both phylogeny and functional needs (Flowers and Colmer, 2008; Flowers et al., 2010). From Table 1, it can be seen that sucrose is more frequently accumulated in monocots than in dicots. Grasses usually accumulate low amount of glycine betaine when this compound is present. In general, species accumulate either quaternary ammonium compounds or proline in response to salinity, but rarely both. Glycine betaine serves as an osmoticum in response to salt stress in most halophytes within the Amaranthaceae. However, the concentration of compatible solutes does not increase with increasing external salinity in all cases, suggesting that the synthesis of these compounds is constitutive in some species; e.g. glycine betaine in *S. fruticosa* (Khan et al., 1998) and β-alanine betaine (at least partially) in *L. latifolium* (Gagneul et al., 2007). In addition, the high concentrations of DMSP in the tissues of *Spartina* species, *W. biflora* and *Saccharum* species would contribute to a high baseline osmotic potential, thus giving constitutive tolerance of salinity-related stress (Otte and Morris, 1994; Stefels, 2000).

**HOW DO OSMOLYTES PROTECT CELL FUNCTION UPON ABIOTIC STRESS?**

The accumulation of osmoprotective compounds represents a specific metabolic response that is important in withstanding harmful conditions (Szabados et al., 2011). Despite the enormous amount of information accumulated in recent decades, the exact function of low-molecular-weight protective compounds in adaptation to extreme environmental conditions is still not completely understood. In this section some important roles of osmoprotective compounds and the advantages of the organic osmolyte system are detailed.

Osmoregulation and sub-cellular compartmentation of osmolytes

From the thermodynamic point of view, osmolytes reduce the osmotic potential of water and thereby the water potential in cells in which they are present (Borowitzka, 1981). In this way, they are able to contribute to osmotic adjustment. The ability for osmotic adjustment via solute accumulation—i.e. an increase in the concentration of a solute in a cell due to an increase in its content rather than a decrease in the water content—has been reported for many plants (Borowitzka, 1981; Yancey et al., 1982). Lowering the water potential is important in the maintenance of tissue water content in the face of low external water potentials generated in saline conditions (Niu et al., 1995). It is clear, however, that osmotic adjustment to the external water potential experienced by halophytes cannot, for
energetic reasons, be achieved by organic solutes alone (Yeo, 1983).

For halophytes in particular, in which a considerable proportion of osmotic adjustment is achieved by ion accumulation, the process seems to be largely dependent on their capacity to compartmentalize ions in the vacuole and to accumulate compatible solutes in the cytoplasm (Flowers et al., 1977, 1986; Wyn Jones et al., 1977; Glenn et al., 1999). Although ion accumulation in the vacuole is relatively simple to establish (Flowers et al., 1986), osmolyte accumulation in the cytosol is more difficult to demonstrate, largely because osmolytes are more difficult to visualize. Glycine betaine has been localized to the cytoplasm of *Suaeda maritima* by staining with iodoplatinic acid (Hall et al., 1978), but histochemical procedures are not available for most solutes. Based on non-aqueous fractionation, Gagneul et al. (2007) revealed that β-alanine betaine and proline were preferentially accumulated in the cytosol in *L. latifolium* after salt treatment, although a vacuolar pool of both solutes could be detected. In addition, isolated protoplasts and vacuoles of cells of *D. spicata* treated with 200 mM NaCl revealed a cytosolic proline concentration estimated to be >230 mM (Ketchum et al., 1991).

An added complication in the evaluation of the role of osmolytes in the adjustment of cytosolic water relations is the possibility that compatible organic solutes move between the cytoplasm and the vacuoles within cells, depending on the osmotic potential of the cytoplasm, without changes in their concentrations at the tissue level (Greenway and Munns, 1980). A model for intracellular compartmentation of inorganic ions and other osmolytes in salinized tissues of higher plants has emerged from the pioneering works of Flowers (1972), Greenway and Osmond (1972), Stewart and Lee (1974) and Wyn Jones et al. (1977). These authors expected that, in plants coping with salinity, the distribution of osmolytes between cell compartments would provide osmotic balance between the cytosol and the vacuole on the one hand and between the cytosol and the apoplastic on the other hand. These approaches have shown that some typical compatible solutes and ions such as Na⁺ and Cl⁻ were indeed preferentially compartmentalized in the extracellular vacuolar compartments and the vacuoles, respectively. However, according to Wyn Jones and Gorham (2002), these statements should now be regarded with caution since great plasticity occurs at both the tissue and the subcellular level. These authors suggested that vacuoles are not inert balloons, but that some of them might be filled with inorganic ions while others might contain large amounts of organic osmolytes. The prevailing model for the allocation of osmolytes at the sub-cellular level might also be modulated by the degree of vacuolation of plant cells, which depends on cell expansion and changes induced by salinity in the volume fraction of the vacuoles (Chang et al., 1996). Compartmental analysis of proline and β-alanine betaine in *L. latifolium* leaf tissues demonstrated that these solutes, mainly located in vacuoles under non-saline conditions, could be partly directed to the cytosol in response to salinization (Gagneul et al., 2007).

Partitioning of proline pools in *Pringplea antiscorbutica* was also shown to be split between the cytoplasm and vacuolar compartments (Aubert et al., 1999). Studies on the C3 chenopods spinach and sugar beet indicate that CMO and BADH are located in the chloroplasts. C4 plants have two types of chloroplast in mesophyll and bundle sheath cells of Kranz-type species. Species having C4 single cells have dimorphic chloroplasts in individual chlorenchyma cells, which are considered specialized for functioning in C4 photosynthesis, analogous to mesophyll and bundle sheath cells in the Kranz C4 system. Thus, it is logical that plants that utilize glycine betaine as an osmoprotectant synthesize it in both types of chloroplast (single-cell C4 and Kranz-type C4 plants), where it is directly available for protecting chloroplasts and for export to protect other parts of the cell (Park et al., 2009).

### Chemical chaperones

One important function of osmoprotective compounds is the stabilization of macromolecular structures (proteins, membranes) under conditions that lead to protein denaturation. Unfortunately, our knowledge of the structure of the cytosol is poor, although it is clear that it is not a dilute solution of proteins and inorganic ions and organic solutes (Cheeseman, 2013). Protective osmolytes improve the thermodynamic stability of proteins by hydrogen bonding, which, in contrast to hydrophobic interactions, does not affect other cellular functions during stress (Kumar, 2009). However, not all osmolytes are equal in the stabilization of proteins (Yancey, 2005). Szabados and Savouré (2010) concluded that proline functions as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes; examples of such roles included the prevention of protein aggregation and stabilization of M4 lactate dehydrogenase during extreme temperatures (Rajendrakumar et al., 1994). This was attributed to the ability of proline to form hydrophilic colloids in aqueous media with a hydrophobic backbone interacting with protein. In addition, proline protects nitrate reductase during heavy metal and osmotic stress (Sharma and Dubey, 2005) and stabilizes ribonucleases and proteases upon arsenate exposure (Mishra and Dubey, 2006). Glycine betaine accumulation protects cytoplasm from ion toxicity, dehydration and temperature stress by stabilizing macromolecule structures, protecting chloroplasts and photosystem II, by stabilizing the association of the extrinsic photosystem II complex proteins and indirectly interacting with phosphatidylcholine moieties to alter their thermodynamic properties (Subbarao et al., 2001).

Polyol hydroxyl groups effectively replace water in establishing hydrogen bonds in the case of cellular dehydration, thus helping to maintain enzyme activities and to protect membrane structures (Noiraud et al., 2001). Trehalose, for example, has the remarkable ability to stabilize membranes and proteins in the dry state (Crowe, 2007). Trehalose was proposed to act as a water replacement, conferring on membranes and proteins physical properties that resemble those seen in the fully hydrated state. Trehalose stabilizes macromolecules and membrane bilayer structure. This effect is achieved via hydrogen bonding between trehalose and polar groups on membrane lipids or proteins, thus maintaining the phase separation of the bilayer and preventing leakage through the membranes. Nevertheless, depending upon concentrations and solvent conditions, naturally occurring osmolytes can also have destabilizing effects on proteins (Singh et al., 2011). For example, trehalose at high concentrations (>1 M) is toxic to...
macromolecules, likely because high viscosity affects dynamic interactions between chaperones and folding substrates and stabilizes protein aggregates. Therefore, it is very likely that cells regulate protein folding, protein disaggregation and protein–protein interactions via concentration-dependent accumulation of osmolytes, which may be an important determinant of adaptation to stress.

By contrast to salt ions, many stabilizing solutes do not bind to proteins; indeed, they are excluded from protein hydration layers (Timasheff, 1992). Termed the ‘osmophobic’ effect by Bolen and Baskakov (2001), exclusion arises from an apparent repulsion between stabilizers and the peptide backbone, explaining how this effect can be universal. Because of this repulsion, proteins will tend to fold more compactly, since this will reduce exposure of the peptide-bond backbone to thermodynamically unfavourable interactions with the stabilizing solute (Yancey, 2005). It has been reported by Paleg et al. (1984) that in Hordeum distichum proline reduces the amount of glutamine synthase precipitated by PEG in a concentration-dependent manner. The authors suggested that these results were consistent with the hypothesis that a protein-containing system in which high concentrations of proline and/or betaine are present is better protected against the biologically unfavourable consequences of dehydration-induced thermodynamic perturbation. Among osmolytes, proline has the property of forming hydrophilic colloids in aqueous media, with a hydrophobic backbone interacting with the protein. On the other hand, the effects of proline might be involved in the hydration layer surrounding phospholipids (Rajendrakumar et al., 1994).

Hydroxyl radical-scavenging activity of osmolytes

Accumulation of ROS is the result of various abiotic stresses, including drought and salinity. The protective function of osmolytes can include the suppression of oxygen radical production, scavenging ROS directly, or contributing to the protection of the enzymes involved in the antioxidant system (Ozgur et al., 2013). Mannitol may scavenge hydroxyl radicals to shield susceptible thiol-regulated enzymes such as phosphoribulokinase plus thioredoxin, ferredoxin and glutathione from inactivation by ROS (Shen et al., 1997). Interestingly, proline was found to be an effective hydroxyl radical scavenger (Smirnoff and Cumbes, 1989).

Proline can act as a singlet oxygen quencher and as a scavenger of OH- radicals (Alia et al., 1997). Thus, proline is an effective quencher of ROS formed under salt, metal and dehydration stress in all plants, including algae (Alia and Sardhi, 1991). Activities of the enzymes catalase, peroxidase and polyphenoloxidase were demonstrated in the algae Thalassiosira pseudonana and Emiliania huxleyi (Sunda et al., 2002), but not yet explored in higher plants. Dimethylsulphonio propane and its breakdown products, e.g. dimethyl sulphide, acrylate, dimethyl sulphoxide and methane sulphonic acid, readily scavenge hydroxyl radicals and other ROS, and thus may serve as an antioxidant system regulated in part by enzymatic cleavage of DMSP (Sunda et al., 2002).

REGULATION OF OSMOLYTE BIOSYNTHESIS BY ENVIRONMENTAL CUES

Osmolyte accumulation can be induced by different stresses, such as salinity, drought, heat and cold (Ghars et al., 2012; Savoure et al., 1997; Slama et al., 2007b, 2008, 2011; DeRidder and Crafts-Brandner, 2008). In order to gain a better picture of their role in adaptation to stress, it is necessary to identify and characterize any signalling pathways involved in their synthesis and their redistribution in planta and/or between subcellular compartments.

How does a plant cell sense changes in osmotic pressure or in ion concentration? In yeast, hyperosmolarity can be sensed by a phosphorelay signal transduction pathway composed of the synthetic lethal of N-end rule (SLN1) His kinase, the tyrosine (Y) phosphatase-dependent phosphorelay intermediate (YPD1) and the suppressor of sensor kinase (SSK1) response regulator, which are homologous to bacterial two-component signal transducers, leading to the activation of the high osmolarity glycerol (HOG1) MAPK pathway to trigger glycerol biosynthesis. The arabidopsis SLN1 homolog histidine kinase (AtHK1) induced by salt and drought stresses has been postulated to be a plant osmosensor (Urano et al., 1999). Recently Kumar et al. (2013) demonstrated that this osmosensor is not involved in ABA, proline and solute accumulation in A. thaliana at low water potential, which calls into questions its role as a main osmosensor at low water potential. Another His kinase, cytokinin response 1 (CRE1), which was identified as a cytokinin receptor, is also able to complement the yeast sln1Δ mutant in the presence of cytokinin (Inoue et al., 2001). Interestingly, Reiser et al. (2003) reported that CRE1 perceives the osmotic signal by turgor sensing, similarly to SLN1. It remains to be shown whether these osmosensors are involved in the regulation of the biosynthesis of osmolytes in plants. At present, how Na+-specific signals are sensed remains elusive in any cellular system. In plants, direct functional connections between members of the salt overly sensitive (SOS) signalling pathway and the dynamics of the cytoskeleton upon salt stress have been established (Wang et al., 2007). The reorganization of the cytoskeleton is a necessary component of cell adaptation to salinity. Further studies should be developed to determine whether the protein(s) responsible for the Na+-specific reorganization of the cytoskeleton may be a constituent of the salinity sensor.

As far as regulation of the biosynthesis of osmolytes is concerned, PSCS and ProDH are the rate-limiting enzymes for the biosynthesis and catabolism of proline, respectively. Proline accumulation is mainly regulated at the transcriptional level of PSCS and ProDH genes during dehydration and rehydration (Szabados and Savoure, 2010). Interestingly, ProDH gene expression is induced by rehydration but is repressed by dehydration. Moreover, its expression is induced by proline and repressed by...
osmotic stress (Yoshiba et al., 1997). In arabidopsis it was reported that the expression of P5CS is independent of ABA upon exposure to cold and osmotic stress, even though expression of this gene can be triggered by treatment with exogenous ABA (Savoure et al., 1997). Recently, lipid signalling pathways have been shown to be involved in the regulation of proline metabolism in A. thaliana (Thiery et al., 2004; Parre et al., 2007). Interestingly, these lipid signalling pathways are regulated in the opposite sense in T. salsuginea, revealing that common signalling components affect the physiology of closely related glycophyte and salt-tolerant plants differently (Ghars et al., 2012).

TRANSPORT OF OSMOLYTES

The allocation and partitioning of compatible osmolyte pools in organs and tissues of halophytic plants is far from being clearly depicted. Osmolyte functions are conceptually associated with accumulation in specific subcellular locations at the right time, thus requiring coordinated transport over both long and short distances.

For polyols, their synthesis as primary products of photosynthesis occurs mainly in the leaves, so the absence of the corresponding synthetic enzymes in sink organs where polyols have been detected is in favour of long-distance transport of these compounds. This has been clearly shown for mannitol and sorbitol (Conde et al., 2011; Noiraud et al., 2001). Functional inositol transporters have been reported in Mesembryanthemum species to take up myo-inositol in a manner depending on the proton gradient, and low Na⁺ concentrations exerted a stimulating effect on inositol movement (Miyazaki et al., 2004).

In contrast, myo-inositol by a stress-induced inositol methyltransferase in a tissue-specific reaction that includes the drastically increased transport of myo-inositol and its derivatives from the leaves to the root system (Nelson et al., 1998).

Proline-specific transporters (ProTs) contribute to tissue- and organ-specific proline deposition under salt stress or during development (Schwacke et al., 1999; Ueda et al., 2007). However, ProTs from tomato (LeProTs) were subsequently shown to also transport glycine betaine as well as proline, although tomato is a betaine non-accumulating plant (Schwacke et al., 1999; Grallath et al., 2005). The barley HvProT1 was reported to recognize proline, but not betaine (Ueda et al., 2001). It has been demonstrated recently that four types of Bet/ProTs could take up choline with higher affinity than betaine, and in situ hybridization experiments in sugar beet indicate the localization of BvBet/ProT1 in phloem and xylem parenchyma cells (Yamada et al., 2011).

EXAMPLES OF IMPROVING PLANT TOLERANCE TO ABIOTIC STRESS BY INCREASING ACCUMULATION OF OSMOLYTES

Using genes from halophytes

A promising approach to avoiding or to reducing crop losses due to the constraints imposed by drought or salinity is to use halophytes instead of glycophytic crops (Kooy and Lieth, 2008). This approach has been long in its gestation (Rozema and Schat, 2013), and the crops generated are often novel (e.g. Salicornia species) and may not be those (e.g. cereals) familiar to most people. Consequently, others have, over the years, advocated the use of resources held in the genomes of halophytes; for example, the contention of some is that they might be bioengineered in related crop species (Amtmann, 2009). However, it is important to point out that wide crossing has not proved to be a very effective route to enhanced salt tolerance of common crops (Yeo and Flowers, 1989). Furthermore, since a large number of genes appear to be involved in tolerance of salt stress, the functions of many of which have yet to be investigated, the genetic engineering of tolerance by single-gene transformation is likely to be unsuccessful in field conditions because plants have to react to a multitude of environmental factors (Flowers and Yeo, 1995). Between 1993 and early 2013 there were nearly 40 papers in which plants were transformed using genes from halophytes; of these papers, nine reported an association with the production of compatible solutes. Two-thirds of the papers described transformation of tobacco, but of these only a single publication reported growth data, and even here the plants were all grown in Murashige and Skoog medium. Of the nine reports of transformations involving compatible solutes from halophytes only a single paper, in which cotton was transformed with choline monoxygenase from Atriplex hortensis, demonstrated that, in a saline field, yield of the transgenics was enhanced over two consecutive years when compared with an untransformed line (Zhang et al., 2009); this enhancement was associated with the enhanced synthesis of glycine betaine. Alternative strategies, such as genomics-based approaches, for halophytes should be considered because they provide access to the genetic basis of salt tolerance and the discovery of superior exotic alleles. Introgression of favourable exotic alleles into elite breeding lines will thereby facilitate the speedy development of superior cultivars.

Using model plants

Recently, the elucidation of the importance of osmolyte accumulation in extremophiles became possible through comparative analysis of closely related halophyte and glycophyte species, especially those that are related to the model plant arabidopsis (Orsini et al., 2010). The halophytes E. salsugineum and C. maritima are close relatives of A. thaliana and can grow under extreme saline conditions (Amtmann, 2009; Debez et al., 2013). Salt cress, E. salsugineum, displays extreme tolerance to high salinity, low humidity and freezing. This member of the Brassicaceae has emerged as a model for understanding the adaptation of halophytes to abiotic stress tolerance due to its homology with the glycophyte model A. thaliana (Amtmann, 2009). Relevant variation in metabolic composition and function in response to salt or drought treatments were observed (Lugan et al., 2010; Pedras and Zheng, 2010). For example, proline contents in E. salsugineum and C. maritima are higher than those in arabidopsis, even in optimal growth conditions, and proline accumulates to higher levels when salt stress is imposed (Ghars et al., 2012; Jdey et al., 2014). Another species that has been frequently used as a model in salt tolerance studies is the facultative halophyte ice plant M. crystallinum, which has bladder cells that accumulate ions (Adams et al., 1998; Cushman and Bohnert, 2000). However, considering the
responses to salinity, none of these dicot plants is ideal as a halophytic model for grain crops, which belong mostly to the Poaceae. Halophytic relatives of wheat (species of Thinopyrum), barley (species of Hordeum) and rice (species of Porteresia) could be investigated as models. However, molecular responses and genome sequence information on these halophyte relatives are poorly documented and need further analysis.

Exogenous application of osmolytes

Exogenous application of osmoprotectants has been reported to have osmoprotective roles in abiotic stress responses and has been suggested as an alternative approach to improving crop productivity under abiotic stress conditions (Ashraf and Foolad, 2007). An improvement in growth after applying exogenous osmolytes has been observed in tobacco, wheat, barley, rice, soybean and common beans (Borojevic et al., 1980; Agboma et al. 1997; Lutts, 2000). In arabidopsis, exogenous application of either proline or glycine betaine triggers the expression of numerous genes involved in regulatory functions and antioxidant defence mechanisms (Oono et al., 2003; Einset et al., 2007). In certain circumstances, however, the accumulation of solutes after exogenous supply can cause deleterious side effects and the universal compatible property of solutes can be questioned (Sulpcie et al., 1998). It remains to be determined what effects the application of osmolytes would have on the growth and survival of halophytes.

IMPORTANCE OF HALOPHYTES AS A SOURCE OF OSMOLYTES FOR PHARMACEUTICAL AND MEDICINAL APPLICATIONS

Osmolytes modulate protein folding and unfolding as well as interactions with lipid membranes and DNA and with ligands that associate and dissociate. The properties of osmolytes are increasingly useful in molecular biology. Proline is currently used in many biological kits with pharmacological and cosmological applications. Osmolytes known to stabilize protein structure improve the crystallization of proteins (Jeruzalmi and Steitz, 1997). Betaine improves cell-free transcription, including PCR (Henke et al., 1997; Hethke et al., 1999), and, in combination with an hyperosmotic medium, it maximizes monoclonal antibody production by hybridoma cells (Welch and Brown, 1996).

Many human pathologies that are linked to poor protein folding, referred to as ‘conformational diseases’, may be corrected by osmolytes (Kim et al., 2006). Proline can protect human cells against carcinogetic oxidative stress (Krishnan et al., 2008). Some osmolytes can also rescue the misfolded protein of cystic fibrosis in vitro (Yancey, 2005). Protein aggregation/misfolding constitutes a hallmark of neurodegenerative pathologies, including Alzheimer’s, Huntington’s and Parkinson’s diseases, and if osmolytes could provide a unifying mechanism of action this may have far-reaching consequences in developing better therapeutic tools for the management of such diseases (Kumar, 2009).

Trehalose has been used for the stabilization of vaccines and liposomes, the hypothermic storage of human organs (Crowe, 2007) and the treatment of dry eye syndrome and dry skin in humans. Trehalose is listed as a prominent ingredient in cosmetics, apparently because it is reputed to inhibit the oxidation of certain fatty acids in vitro that might be related to body odour (Crowe, 2007). These various uses suggest there may be many opportunities to exploit halophytes for the diversity of osmolytes they contain.

CONCLUSIONS

Environmental stress factors affect plant growth and constitute a growing threat to sustainable agriculture. Upon exposure to abiotic stress, it is commonly observed that, concomitantly with controlled ion uptake and sequestration of toxic ions into the vacuole, halophytes also synthesize and accumulate metabolites that are compatible with metabolic reactions and even stabilize cellular structures, photosynthetic complexes, specific enzymes and other macromolecules. Compatible osmolytes include a variety of compounds such as sugars, amino acids, tertiary sulphonium compounds, quaternary ammonium compounds and polyhydric alcohols. These osmolytes do not have any harmful effects on plant metabolism even at high concentrations in the cytoplasm.

Halophytes constitute an interesting source of various osmolytes (Table 1). Interestingly, certain osmolytes, such as nitrogen compounds and carbohydrates, are typical of certain families. Investigation of new halophytic species, especially those growing in extreme environments, is of importance in order to find new compounds. These molecules could be very useful in the pharmaceutical industry and in medicine due to their ability to stabilize proteins and to protect against oxidative damage.

Halophytes also represent an important genetic reservoir that can be exploited for improving crops for marginal land. Engineering osmoprotectant biosynthesis pathways is a potential way to improve stress tolerance. First-generation engineering work, much of it with single genes, has successfully introduced osmoprotectant pathways into plants that lack them naturally. However, engineered osmoprotectant levels are generally low and the increases in tolerance commensurately small. For these reasons, simultaneous engineering of osmolyte accumulation and various other traits by transgenic technology is a feasible strategy to improve abiotic stress tolerance in crops.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: osmolyte distribution in representative halophyte species among angiosperms.

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concerning the distribution and production of osmoprotective compounds in halophytic plants.

LITERATURE CITED


