BioMol Concepts, Vol. 2 (2011), pp. 407–419 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/BMC.2011.032

Review

Ion transport and osmotic adjustment in plants and bacteria

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Abstract

Plants and bacteria respond to hyperosmotic stress by an increase in intracellular osmolality, adjusting their cell turgor to altered growth conditions. This can be achieved either by increased uptake or *de novo* synthesis of a variety of organic osmolytes (so-called 'compatible solutes'), or by controlling fluxes of ions across cellular membranes. The relative contributions of each of these mechanisms have been debated in literature for many years and remain unresolved. This paper summarises all the arguments and reopens a discussion on the efficiency and strategies of osmotic adjustment in plants and bacteria. We show that the bulk of osmotic adjustment in both plants and bacteria is achieved by increased accumulation of inorganic osmolytes such as K^+, Na^+ and Cl^-. This is applicable to both halophyte and glycophyte species. At the same time, *de novo* synthesis of compatible solutes is an energetically expensive and slow option and can be used only for the fine adjustment of the cell osmotic potential. The most likely role the organic osmolytes play in osmotic adjustment is in osmoprotection of key membrane transport proteins and reactive oxygen species (ROS) scavenging. The specific mechanisms by which compatible solutes regulate activity of ion transporters remain elusive and require more thorough investigation. It is concluded that creating transgenic species with increased levels of organic osmolytes by itself is counterproductive due to high yield penalties; all these attempts should be complemented by a concurrent increase in the accumulation of inorganic ions directly used for osmotic adjustment.

Keywords: compatible solutes; inorganic ion; osmolyte; osmosensing; potassium; ROS scavenging; sodium.

List of abbreviations

ROS, reactive oxygen species; ABA, abscisic acid; QTL, quantitative trait loci; SOS, salt overly sensitive.

Introduction

Osmotic stress is ubiquitous in nature and has detrimental effects on all living organisms, from higher plants to bacteria and fungal species. It may come about in a range of environmental conditions and can result in severe disturbance to the cell's metabolism, growth and survival. Plant dehydration as a result of drought is a classical example of osmotic stress, with the shoot growth rate being arrested almost immediately after stress onset, and restored, albeit at much slower rates, within a couple of hours (1). The economic penalties of this growth reduction are in the billion dollar range. Osmotic stress is also one of the major components of salinity stress. The latter severely limits global agricultural productivity, rendering an estimated one third of the world's irrigated land unsuitable for crops (2, 3).

Both drought and salinity result in hyperosmotic stress being imposed on the organism. At the other end of the spectrum is hypoosmotic stress. A large number of organisms, from higher plants to unicellular organisms inhabiting terrestrial waters, are often exposed to frequent variations in osmolarity during their life cycle. Consequently, they are expected to have developed an adaptive mechanism to be osmotically adjusted to the changing environment. This is done by increasing intracellular osmolality in response to hyperosmotic stress, or by decreasing it in response to hypoosmotic stress, to adjust the cell turgor to altered growth conditions. This review addresses responses to hyperosmotic stress only.

In order to adjust to increased external osmolality, cells in all three kingdoms accumulate a variety of molecules in the cytoplasm. These counteract the external osmotic pressure. Three major avenues are available for organisms. First, cells can accumulate a range of organic osmolytes (so-called compatible solutes) by increasing their uptake from external media. This approach is widely used by food-dwelling microorganisms such as bacteria or yeast (4, 5), which often have ample supply of such compatible solutes in the growth media. Bacterial cells are known to possess a range of transporters mediating uptake of various compatible solutes (6–8), and the expression of these transporters is known to be significantly increased under hyperosmotic stress conditions (9, 10). The second option for osmotic adjustment is a *de novo* synthesis of such compatible solutes within the cell. This option is used by organisms with no, or very limited, access to the external pool of organic osmolytes. Plants are a classical example, with the onset of hyperosmotic stress resulting in activation of genes responsible for organic osmolyte biosynthesis and leading to an accumulation of signifi-

cant amounts of glycine betaine, proline and polyols under either drought or salinity conditions (11, 12). Finally, the third option is to accumulate more inorganic ions (mainly Na^+, Cl^- and K^+) for osmotic adjustment purposes.

The relative contribution of each of these mechanisms and, specifically, the preference for organic vs. inorganic osmolytes, is still rigorously disputed in the literature. A plant's ability to accumulate compatible solutes is frequently cited as a key putative mechanism for increasing the yield of crops subjected to osmotic stress (13), and significant efforts of both plant breeders and molecular biologists are invested into attempts to improve plant osmotic stress tolerance via manipulation of the biosynthetic pathways of compatible solutes (e.g., (14–16); discussed in detail below). Furthermore, an almost unchallenged view prevails that accumulation of organic solutes is a prerequisite for osmotic adjustment in all non-halophilic microorganisms (4, 17).

This paper challenges this view and reopens a discussion on the efficiency and strategies of osmotic adjustment in plants and bacteria. We analyse the available literature and argue that organic osmolytes accumulation cannot directly contribute to conventional water retention and osmotic adjustment in cells. We argue that inorganic ions provide the bulk of osmotic adjustment, and that the physiological relevance of stress-induced increase in the levels of compatible solutes in plants and bacteria is in the control of activity of key ion transporters by either direct protection of transport proteins (chaperon function) or by scavenging ROS and preventing oxidative stress-induced disturbance to intracellular ionic homeostasis.

Classical concepts and dogmas

Compatible solutes are defined as small water-soluble molecules that may be accumulated in cells at high concentrations without affecting metabolic reactions in either the cytosol or major organelles (12, 18). Four major classes of osmolytes are usually distinguished (19): 1) sugars (e.g., sucrose or trehalose); 2) polyols (e.g., glycerol, sorbitol or mannitol); 3) amino acids (e.g., glutamate or proline); and 4) quaternary ammonium compounds (e.g., glycine betaine). According to the classical view, accumulation of these non-toxic (thus compatible) osmotically active solutes will result in an increase in cellular osmolarity leading to the influx of water into, or at least reducing the efflux from cells, thus providing the turgor necessary for cell expansion (6, 19–22). The above concept became a dogma and has dominated the literature for the past two decades.

Both plants [reviewed in (11, 23, 24)] and bacteria (6, 22) dramatically increase the amount of organic osmolytes under hyperosmotic stress conditions. This increase depends strongly on the severity of the stress, and may range from factors of several to hundreds. Furthermore, a correlation between the amount of compatible solute and osmotic stress tolerance has been widely documented [reviewed in (11, 25)]. Supportive evidence also comes from experiments with exogenous application of osmolytes (26, 27). As a result, osmolyte accumulation has long been emphasised as a selection criterion in traditional crop breeding programs (28–33), and numerous genetic engineering attempts have been made to manipulate the biosynthetic pathways of compatible solutes in order to enhance osmotic stress tolerance (14, 23, 26, 27, 34–37). Some of these are summarised in Table 1. The main attraction of this approach is the fact that osmolyte accumulation is often controlled by only one gene (13), making it easy to manipulate. This also opens up prospects to identify molecular markers and QTLs linked to a plant's capacity to accumulate osmolytes.

Table 1 Selected examples of genetic manipulation in the biosynthesis pathway of compatible solutes in order to enhance salt tolerance in plants.

Osmolyte targeted	Gene	Species
Proline	*P5CS*	Rice
	NhaA	Rice
	ProDH	Tobacco
Glycine betaine	*codA*	Rice
	badH	Rice
	betA	Tobacco
Mannitol	*mtlD*	Tomato
	mtlD	Wheat
	mtlD	Rice
Trehalose	*TPS1*	Maize
	TPS1	Alfalfa
	otsA-otsB	Rice
Fructans	*sacB*	Tobacco
Inositol	*imt1*	Tobacco

Problems and issues

In recent years, the conventional role of compatible solutes as osmolytes has been seriously questioned. It appears that most evidence to support the contribution of compatible solutes to cell adaptation to dehydration stress remains circumstantial (25, 38), and there are many controversies in the literature arguing against the direct involvement of organic osmolytes in the maintenance of cell turgor. Some of these arguments are summarised below.

Argument 1: concentrations are too low

From a thermodynamical point of view, concentrations of organic osmolytes are often far too low for conventional osmotic adjustment. Taking halophyte plant species as an example, the two major known osmolytes are glycine betaine and proline (39, 40). Their reported concentrations under stress conditions range from 3–10 mM (41, 42) to 120–150 mM (39). Even assuming that these compatible solutes are located exclusively within the cytosol, these concentrations are hardly sufficient for full osmotic adjustment, given the reported Na^+ concentrations in the vacuole [e.g., 500–600 mM; (43)] or external media (200–500 mM) found under typical conditions.

The same arguments are applicable to crop species. Proline concentrations range between 2 and 6 mM when measured in *Arabidopsis*, pea or rice tissues in response to NaCl treatment (44–46). Even assuming all of it is contained in the cytosol and plastids (which together comprise 10–15% of the total volume), this will amount to <50 mM in concentration. One to eight mM proline levels were reported in transgenic tomatoes under stress conditions (47); most of it was located in chloroplasts. Even lower (5 mM) trehalose levels were reported in rice cell cytosol (15). These numbers are far too low for conventional water retention under stress conditions.

Bacterial systems also appear to be no exception from the above controversy. The amount of betaine found in *E. coli* and *Staphylococcus epidermidis* was about 90 mM and could hardly directly assist in osmotic adjustment to 3% (over 500 mM) NaCl (48). In *Methanohalophilus* bacteria, the overall contribution of compatible solutes to cell osmolality was <50% for organisms grown under high (1.4 to 2.7 M) salinity even when organic osmolytes were present at high (0.5 M) concentrations in the basal media (48). In the absence of compatible solute in the media, the overall contribution of five major compatible solutes found in this organism was <20% (0.54 M in total for the organism grown in 2.1 M NaCl).

Argument 2: high cost of osmolyte production

With the possible exception of food-dwelling microorganisms, the concentration of organic osmolytes in the external environment is usually far too low to rely upon for osmotic adjustment. Hence, plants and bacteria have to synthesise these compatible solutes *de novo*. This comes at a very high cost to the organism. Raven (49) estimated that, in vascular plants, the photon cost to synthesise one mole of compatible solute ranges between 67 and 99 moles of photons. At the same time, only four to seven moles of photons are needed to generate osmolality using KCl or NaCl. Similar calculations were done by Oren (50) for bacterial cells who showed that between 30 and 109 molecules of ATP appear to be required for the autotrophic biosynthesis of one molecule of a different compatible solute (23–79 for the heterotrophs). At the same time, one molecule of ATP should suffice for the accumulation of two K^+ and two Cl^- ions (50). Hence, the energy cost of organic osmolytes production is at least 10 times higher compared with inorganic ion uptake. Such high carbon cost results in substantial yield penalties. In this context it should be noted that genotypes of high osmotic adjustment capacity under drought usually have a relatively low yield potential under non-stressed conditions (31, 51). A similar trade-off between plant yield penalties under control conditions and abiotic stress tolerance has also been reported for salinity tolerance (52). This is further illustrated in Figure 1 using an extremely halophilic methanogen *Methanohalophilus* as an example. This extremophilic organism can grow at extremely high (up to 4 M NaCl) salinities. As illustrated in Figure 1, *de novo* synthesis of zwitterionic compounds (glutamine, N6-acetyl lysine, and glycine betaine) used for osmotic adjustment strongly and negatively correlate with bacterial growth.

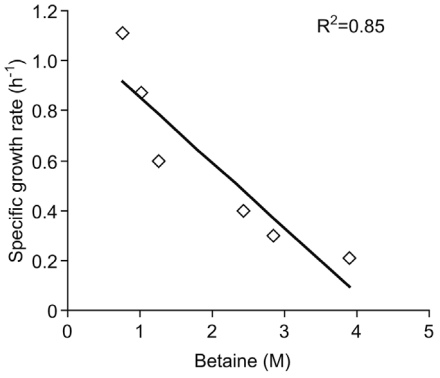

Figure 1 Negative correlation between organic osmolyte production and specific growth rate found in extremophylic bacteria *Methanohalophilus*.
The results shown are recalculated based on data reported by Lai et al. (144).

Argument 3: the process is too slow

Synthesis of compatible solutes is a rather slow process, operating on a timescale of hours and days. At the same time, many organisms may experience much more rapid fluctuations in media osmolality and, hence, require faster paths for osmotic adjustment. Organisms inhabiting coastal areas (and, hence, regularly inundated by saline water at high tide and then diluted by frequent rains) may be used as an example (Figure 2). Rapid turgor recovery may also be essential for plants which experience acute water stress in natural conditions throughout ontogeny (such as with a shallow root system). Direct measurements of kinetics of epidermal cell turgor recovery by microelectrode pressure-probes have revealed that Arabidopsis roots are able to regain >90% of their turgor within 40 min of the onset of hyperosmotic stress (53). Earlier Bisson et al. (54) showed that 25% of initial turgor in *Chara longifolia* was recovered within 5 min, and the remaining balance of turgor pressure was regained during a slower phase within 40 min. Obviously, rapid turgor recovery cannot be attributed to organic osmolyte production.

Argument 4: the lack of correlation between stress tolerance and amount of organic osmolytes

Arguably the most important controversy is due to the lack of a consistent correlation between osmotic stress tolerance and accumulation of organic osmolytes. Arabidopsis *rss* mutants with reduced salt sensitivity show a much lower capacity for proline accumulation under both salinity and osmotic stresses compared with wild types (55). Salt-sensitive genotypes often accumulate much more organic osmolytes compared with tolerant varieties [e.g., wheat (56); tomato (57); rice (58); barley (59)]. This is further illustrated in Figure 3, which summarises the relative contribution of organic osmolytes towards osmotic adjustment in barley

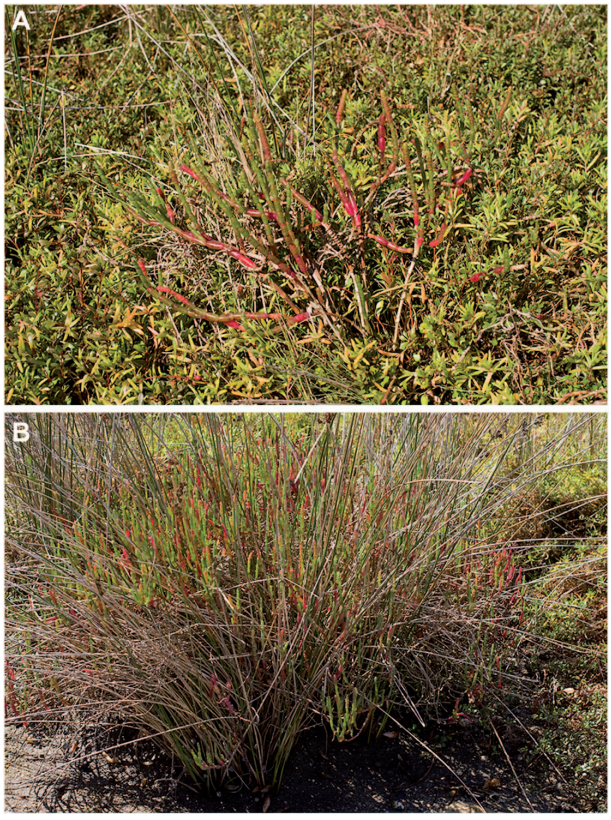

Figure 2 Plant species inhabiting sea cost of North-East Tasmania and subject to the frequent (12 h periodicity) inundation with saline water.
(A) *Sarcocornia quinqueflora* (beaded glasswort); (B) *Juncus kraussii*. To maintain their growth, these organisms must rapidly adjust the root and shoot osmolality to rapidly fluctuating environment. Photo courtesy of Paul Sakov.

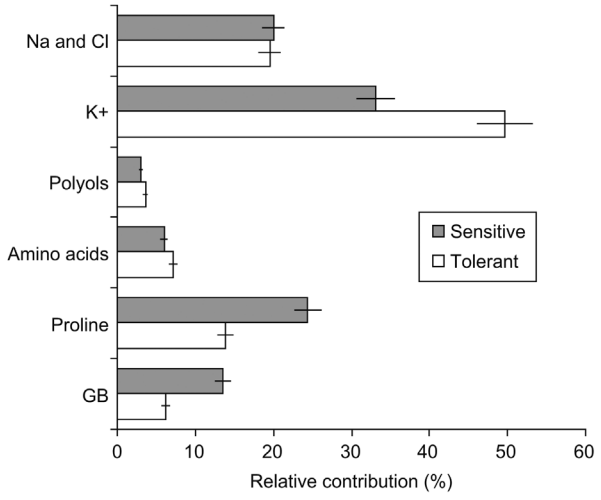

Figure 3 Relative contribution of inorganic and organic solutes towards leaf osmotic adjustment in barley genotypes contrasting in their salinity tolerance [based on (59)].
Plants were exposed to 320 mM NaCl stress for 4 weeks.

genotypes that exhibit contrasts in salinity tolerance. Sensitive varieties accumulate about twice the amount of proline and glycine betaine, while no significant ($p<0.05$) difference is found for polyols and amino acids other than glycine betaine (Figure 3). Thus, although both sensitive and tolerant varieties are able to osmotically adjust themselves, salt-sensitive varieties consume about 4.5 times more ATP for synthesising glycine betaine and proline compared with salt-tolerant genotypes (59). This will come with severe yield penalties and explain a huge difference in plant performance under saline conditions between these varieties (59, 60). This poses a question: could the reduction in growth rate observed in plants immediately after the onset of hyperosmotic stress in crops (1) be caused by the dramatic shift in the energy balance and the redrawing of (almost) all available ATP towards osmolyte biosynthesis, at the expense of all other metabolic processes?

Argument 5: are they really compatible?

The entire concept of osmotic adjustment via accumulation of organic osmolytes is based on the assumption that an increase in osmolyte concentration should not interfere significantly with cell metabolism (11, 18); hence, their definition as *compatible* solutes. However, several papers have reported that accumulation of large amounts of proline in stressed plant tissues causes toxicity effects [e.g., (61)]; similar observations were made for exogenous proline application to non-stressed plants (62, 63). Hence, the above assumption of organic osmolytes not interfering with cell metabolism needs to be treated with some caution.

Inorganic ion uptake: a viable alternative

Theoretical considerations

Expensive or not, organisms must adjust their osmotic potential to function normally under environmental constraints. For plants, the lack of osmotic adjustment will result in the loss of turgor and, hence, the lack of growth. Moreover, such osmotic adjustment must occur really fast. Indeed, species inhabiting costal areas shown as an example in Figure 2 will require a constant and rapid (within 1–2 h) readjustment of cell turgor, assuming organisms want to maintain active growth rather then just survive. Thus, *de novo* synthesis of compatible solutes (the process that usually take over 10 h at the very least) is hardly an option here. Interestingly, there are reports showing that the concentration of organic osmolytes may undergo very significant [e.g., 5-fold (64)] diurnal fluctuations. However, given the fact that these have been observed in land species not experiencing the above regular changes in external osmolarity, it is unlikely that such fluctuations are needed for osmotic adjustment. More likely, such dramatic variation may be required to match the amount of ROS produced during day/night fluctuations. This issue is discussed in more detail below.

A viable alternative to the energetically expensive and rather slow process of osmolyte biosynthesis is osmotic

adjustment by means of inorganic ions. Indeed, from thermodynamical point of view water retention within the cell may be achieved equally well by increased concentration of both organic and inorganic molecules. There is one caveat here, however. Such an increase in osmolyte concentration should not interfere significantly will cell metabolism. As discussed above, this is not always the case for organic osmolytes. What about inorganic ions?

Three major candidates to be considered are K^+, Na^+ and Cl^-. Of these, potassium is the most cytosolically abundant ion; it can range from 100–150 mM in plants (65) to as much as several moles in some bacteria (50). Thus, potassium is ideally suited for this purpose. The only problem is that, under stress conditions, the electrochemical gradients favour potassium *loss* [see (3) for a review] and, hence, organisms will have to rely on high affinity K^+ uptake, which may be a slow and energetically expensive option. Two other ions, Na^+ and Cl^-, are plentiful in the external media (especially under saline conditions). Of these, Na^+ can enter the cell passively and, hence, be used as cheap osmotica to maintain normal cell turgor. There is a big 'but', however: Na^+ ions are toxic and can cause severe disruptions to cell metabolism. With a few possible exceptions for extremophylic bacteria (50), enzymatic activity appears to be substantially inhibited by elevated cytosolic Na^+ levels, regardless of whether this occurs in halophyte or glycophyte species (3). The imbalance in cytosolic K/Na ratio has a dramatic impact on cell metabolism and may trigger programmed cell death (66). Thus, efficient sequestration in the vacuole is absolutely essential. Even so, to pump one mole of Na^+ against the electrochemical gradient into the vacuole takes only 3.5 mol of ATP compared with 30–50 mol of ATP required to produce one mole of organic osmolyte (49). Thus, the energetic benefits are obvious. This is also true for bacteria: the overall cost of the establishment of the ion gradients by transport of inorganic ions after osmotic upshock is much lower than that required to fill the cytoplasm with organic osmotic solutes (50).

To maintain membrane electroneutrality, increased K^+ uptake should be accompanied by either the equivalent loss of cations (which would negate the osmotic effect) or the accumulation of anions. Chloride ions appear to be suited for this role. With few exceptions (e.g., citrus or grapevines for higher plants), accumulation of Cl^- in the cytosol does not appear to have major detrimental effects on cell metabolism. Hence, accumulation of K^+ can be accompanied by increased Cl^- uptake without serious metabolic consequences. Uptake of Cl^-, another inorganic osmolyte, will also double the osmotic effect and require less K^+ to be taken.

It should be added that in higher plants, Na^+ loading into the xylem is most likely a metabolically active process that is mediated by SOS1 Na^+/H^+ exchangers at the xylem-parenchyma interface (2, 67). It is then natural to ask why plants invest into active loading of the ion that is toxic to their metabolism? The most likely explanation is that they need it in the shoot to maintain cell turgor and, ultimately, shoot growth. Supporting this idea are observations that xylem sap Na^+ concentrations were substantially higher in salt-tolerant barley varieties compared with salt-sensitive genotypes (67).

Experimental evidence

Higher plants The idea that changes in ion fluxes in response to osmotic stress may provide quick (within a few minutes) osmotic adjustment and maintain normal turgor is rather old [e.g., (68)]; however, direct experimental evidence is scant and mostly based on theoretical calculations of changes in cell osmotic potential caused by measured fluxes of inorganic ions (69, 70). The first direct support at cellular level came from concurrent measurements of net ion fluxes in osmotically-stressed *Arabidopsis thaliana* epidermal root cells [measured by the microelectrode ion flux estimation (MIFE) technique] and cell turgor changes (measured by the pressure-probe technique) (53). It was shown that over 90% of cell turgor was recovered by uptake of three major inorganic ions (K^+, Na^+ and Cl^-) within 40 min of the onset of hyperosmotic stress. Very similar numbers were obtained for bean mesophyll cells (71).

The role of inorganic ions in plant osmotic adjustment may be validated relatively easily at the whole plant level by direct measurements of root or shoot osmolality and comparing it with the contribution of K^+, Na^+ and Cl^- measured in the tissue sap. A recently published comprehensive study including 50 bread and durum wheat varieties has found that between 87 and 100% of shoot sap osmolality was achieved by using just K^+, Na^+ and Cl^- (72). Potassium made a major contribution ($\sim$63%) towards osmotic adjustment in shoot cells under control conditions in both durum and bread species, and shoot sap K^+ increased significantly in nearly all durum and bread wheat genotypes under salt stress conditions (72). However, as the cell osmolality had to increase by $\sim$2.5-fold to enable osmotic adjustment under hyperosmotic stress conditions, potassium alone can't meet this demand, and plants had to accumulate significant amounts of Na^+ and Cl^-. Consistent results were also obtained for barley (59). The relative contribution of Na and Cl towards the osmotic adjustment did not differ significantly between salt-tolerant and salt-sensitive genotypes (Figure 3), but contribution of K^+ towards cell osmotic adjustment in the cytosol was 50% in salt-tolerant varieties, while in salt-sensitive varieties it was only 33% (Figure 3). Thus, it appears that sensitive genotypes invest about 4.5 times more energy towards osmolyte biosynthesis than tolerant genotypes. As commented above, this comes with severe yield penalties and explains the huge difference in plant performance under saline conditions between these varieties (59, 60).

Several more examples come from halophyte species. It is widely accepted that in halophytes cell turgor is maintained by storage of Na^+ and Cl^- in vacuoles, with the solute potential of the cytosol being adjusted by accumulation of K^+ and organic solutes (39, 73, 74). According to Glenn et al. (74), the three major inorganic ions, Na^+, K^+ and Cl^-, account for 80 to 95% of the cell sap osmotic pressure in both halophyte grasses and dicots. Our recent experiments with *Chenopodium quinoa* show that over 95% of cell osmotic turgor in old leaves, and between 80 and 100% of cell turgor in

young leaves, may be adjusted by means of accumulation of Na^+ and K^+ only; the rest is attributed to chloride (75). It is not surprising, therefore, that halophytes accumulate substantial amounts (>10% of dry weight each) of Na^+ and Cl^- in their shoots (76).

Algae It has long been known that salt tolerant *Lamprothamnium* sp regulates its turgor in salinities up to seawater level, and that this process is achieved by altered uptake of inorganic ions, including Na^+. Both fluxes and vacuolar concentrations of inorganic ions were measured in *Lamprothamnium* sp as a function of either hyper- or hypo- osmotic stress (77–79). Similar findings were also reported for some other Characeae species (54, 80). Moreover, ion channels *per se* were suggested to operate as osmosensors in the transduction of pressure signals that lead to osmotic adjustment in these species (81).

Bacteria and fungi In non-halophilic bacteria, potassium is the most abundant inorganic ion in the cell cytosol, with a typical concentration of ~200 mM; (82). It therefore seems to be the most suitable candidate for osmotic adjustment purposes (83). Increased cellular accumulation of K^+ has been claimed to occur in response to a large number of ionic and non-ionic osmotica (84–86). Experiments conducted in our laboratory show that intracellular K^+ levels in *E. coli* increased from 180–200 mM to 280 mM upon 2% NaCl treatment (87), and dropped dramatically to as low as 100 mM in cells grown at 10% NaCl. At the same time, internal Na^+ concentration increased from several mM at low NaCl concentrations to over 1000 mM at 10% NaCl (87). Interestingly, the 2% NaCl treatment appeared to be the most beneficial to bacterial cell growth, and model calculations have revealed that, for this treatment, the osmotic balance in bacterial cells was achieved almost exclusively by increased uptake of K^+ (88). As soon as they started to lose K^+ due to NaCl-induced membrane depolarisation, bacterial cells had to rely more extensively on Na^+. With no vacuole to sequester it away from metabolically active pathways, this resulted in a gradual decline in cell viability and reduced yield (87).

Very consistent results are reported for halobacteria. As shown in Table 2, potassium contributes almost exclusively to osmotic adjustment in extremely tolerant halobacteria such as *H. saccharovorum* and *H. trapanicum* and makes a major (over 70%) contribution towards osmotic adjustment in moderately tolerant species. Amazingly, in some organisms intracellular K^+ concentrations can be as high as 5 M (89).

Non-invasive ion-selective microelectrode technique was also used to elucidate the ionic mechanisms of osmotic adjustment in the marine protist thraustochytrid. Hypoosmotic stress caused significant efflux of Na^+, Cl^- and K^+ from thraustochytrid cells (90). Model calculations showed that almost complete osmotic adjustment was achieved within the first 30 min after stress onset. Of these, sodium was the major contributor (more than half of the total osmotic adjustment), with chloride being the second major contributor (90). The role of K^+ in the process of osmotic adjustment was relatively small, which is hardly surprising for a marine organism used to living in a Na-rich environment. Importantly, thraustochytrid cells showed normal growth patterns even when grown in a sodium-free solution, provided the medium osmolality was adjusted with mannitol to a level equivalent to seawater's. This suggests that the need for sodium in the thraustochytrid growth cycle is due to its role in cell osmotic adjustment rather than because of the direct Na^+ involvement in cell metabolism. These results are consistent with theoretical calculations by Wethered and Jennings (91) who suggested that inorganic ions made the major contribution to solute potential of *Thraustochytrium* spp. sporangia, and estimated the contribution of organic solutes to be insignificant. Contribution of inorganic ions (Na^+, Cl^- and K^+) was also found to be essential for osmotic adjustment in yeast (92), algae (93, 94), and marine fungi (95).

A direct comparison between cell turgor recovery and kinetics of net ion fluxes was made in filamentous fungus *Neurospora crassa* in response to hyperosmotic stress (96). Turgor recovery was complete within 60 min and occurred concurrently with net K^+ and Cl^- uptake. The magnitude of the ion uptake was more than sufficient to account for the osmotic gradients required for turgor, ruling out a need for any organic osmolyte involvement. Interestingly, *os-1* and *os-2* osmotic mutants with altered kinetics of turgor recovery also showed different ion flux patterns compared to wild types (96). Taken together, this strongly emphasises the dominant role of inorganic ion uptake for osmotic adjustment in this organism.

What then is the role of organic osmolytes?

While it appears that compatible solutes play a very limited role in conventional water retention and are not directly involved in osmotic adjustment, they nonetheless are fundamental for the cells' adaptive responses to osmotic stress.

Table 2 Intracellular potassium concentrations and their contribution to osmotic adjustment in some halobacteria [based on (143)].

Organism	Tolerance	Intracellular K, M	% contribution
H. halobium	Extreme	3.0	96
H. saccharovorum	Extreme	3.4	100
H. trapanicum	Extreme	3.2	100
H. volcanii	Moderate	2.1	68
H. mediterranei	Moderate	1.9	71
H. gibbonsii	Moderate	2.3	74

Cells were grown in a media containing 17% NaCl.

Multiple functions have been suggested, including their role as low-molecular-weight chaperones; in membrane integrity maintenance; protecting the structure of enzymes and proteins; PSII protection and repair; redox potential buffering; and ROS scavenging (11, 12, 21, 97–100). Importantly, there may be more than one function for a particular osmolyte, and different compatible solutes could have different functions (101, 102). Even more importantly, these functions do not require high amounts of organic osmolytes and, hence, do not come at a high energetic cost to the organism. Some of these issues are discussed in more detail below in the context of plant osmotic adjustment.

Hyperosmotic stress leads to increased levels of ROS in both higher plants (2, 103) and bacteria (104, 105), and the ability of osmolytes to scavenge these ROS is widely reported (11, 97, 106), at least *in vitro*. Also reported is a correlation between oxidative stress tolerance and both salinity (107, 108) and drought (109) stress tolerance in plants. The scavenging role appears to be specific for each particular osmolyte. While all polyols are known to be active ROS scavengers, glycine betaine seems to be rather inefficient in this role (97, 110). Moreover, there appears to be a rather high specificity in the ability of compatible solutes to scavenge a particular ROS. As such, mannitol has been reported to be active against hydroxyl radicals and not against hydrogen peroxide or oxygen radical (47). Importantly, ROS scavenging does not require high concentrations of accumulated osmolytes and can be achieved at physiologically relevant concentrations. This also explains preferential accumulation of these osmolytes in specific organelles such as chloroplasts or mitochondria (111). In this context, mannitol at concentrations <100 mM in chloroplasts specifically reduces damage by hydroxyl radicals generated through the Fenton reaction between free Fe^{2+} and H_2O_2 (47, 99, 106, 112).

How does this relate to osmotic adjustment? As discussed above, both thermodynamically and metabolically potassium is the most preferred osmolyte to be used for osmotic adjustment by cells of any origin. However, under hyperosmotic stress conditions, massive K^+ efflux has been observed from both root (3, 59, 60, 75, 113) and leaf (71, 114) tissues in higher plants; similar results were reported for bacterial systems (87). In plants, this K^+ efflux is mediated by two types of transport systems: (1) outward-rectifying K^+ channels activated by membrane depolarisation under saline conditions; and (2) ROS-activated K^+-permeable non-selective cation channels (3, 115). This potassium efflux results in depletion of the intracellular K^+ pool (116, 117), imposing at least two hurdles on cells. First, the requirement to maintain cytosolic K^+ homeostasis for metabolic purposes rapidly depletes the vacuolar K^+ pool and results in turgor loss. Second, unavailability of K^+ for osmotic adjustment purposes attributes this role to either Na^+ (hence, toxicity issue) or *de novo* synthesised organic osmolytes (hence, energy cost). Thus, the ability of compatible solutes to scavenge ROS may be essential to retaining cytosolic K^+, assisting plants in osmotic adjustment.

Recent experiments in our laboratory have shown that some compatible solutes are very efficient in reducing the extent of K^+ loss in response to both salinity (118, 119) and oxidative stress (120). Exogenously supplied physiologically relevant (5 mM) concentrations of proline and glycine betaine rapidly ameliorated NaCl-induced K^+ efflux from barley roots (118). Further experiments have shown that 21 (of 26) protein- and non-protein- amino acids caused significant ($p<0.05$) mitigation of NaCl-induced K^+ efflux, while two amino acids (valine and ornithine) substantially enhanced the detrimental effects of salinity on K^+ homeostasis. These mitigating effects were obtained *in situ* at physiologically relevant (0.1–1 mM) concentrations (119). Hence, plants do not need to synthesise substantial quantities of organic osmolytes to control intracellular ionic homeostasis. It should be added that, to the best of our knowledge, all previous reports of stabilising effects of amino acids on membrane permeability and enzymatic activity were obtained *in vitro* and for physiologically unrealistic [e.g., 100 to 500 mM (121, 122)] concentrations. Based on these results, the following model is suggested (Figure 4). Onset of hyperosmotic stress triggers immediate activation of activity of major ion transporters leading to a rapid accumulation of inorganic osmolytes and osmotic adjustment. The onset of stress also leads to a progressive accumulation of ROS in the cell. This has a detrimental effect on the activity of ion transporters and, specifically, K^+ retention in the cell (3, 115). Hyperosmotic stress also triggers de novo synthesis of compatible solutes via stress-induced gene expression; these compatible solutes are then used to scavenge ROS and prevent their detrimental impact on ion homeostasis. They also act as molecular chap-

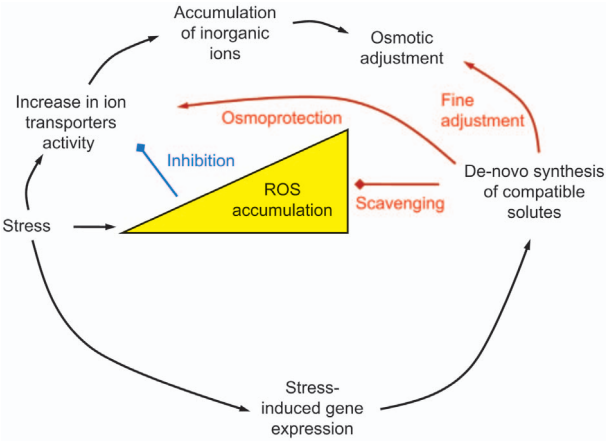

Figure 4 The suggested model of involvement of organic osmolytes in osmotic adjustment in living organisms.
Onset of hyperosmotic stress results in a rapid accumulation of inorganic osmolytes and osmotic adjustment. The onset of stress also leads to a progressive accumulation of ROS in the cell which has a detrimental effect on activity of ion transporters. *De novo* synthesis of compatible solutes via stress-induced gene expression is also triggered. These compatible solutes are used then to scavenge ROS and prevent their detrimental impact on ion homeostasis. They also act as molecular chaperons for key ion transporters and contribute to fine osmotic adjustment in the cell (predominately, in cell cytosol).

erones for key ion transporters, and contribute to fine osmotic adjustment in the cell (predominantly in the cell cytosol).

Where are we now?

Overall, it appears that 'compatible solutes' are not as 'compatible' as initially believed; moreover, they unlikely play a direct role in conventional water retention. The levels of compatible solutes accumulated in transgenic plants are not high enough to be osmotically significant; their biosynthesis is slow and cannot cope with the organism's demand for rapid osmotic adjustment; and the energetic cost of osmolyte biosynthesis is substantial. The bulk of osmotic adjustment in both plants and bacteria is achieved by the increased uptake and retention of inorganic ions. Amazingly, until now the efforts of plant breeders had focused on either selecting or engineering plants with efficient Na exclusion from uptake (1, 2, 123, 124). Under these circumstances, the only remaining option for plant osmotic adjustment was increased osmolyte biosynthesis, with all the associated costs. It is no wonder that the practical outcomes of this approach are very limited.

It also appears that organic osmolytes have very specific and complex roles, such as PSII protection and repair, membrane and enzyme protection, ROS scavenging, energy sinks and redox potential buffering, the use of osmolytes as a source of N and C during the recovery from stress, signalling, and control over ion transport and partitioning (3, 21, 23, 35). Importantly, none of these roles require a very substantial accumulation of osmolytes to have a beneficial effect. Hence, all the attempts of molecular geneticists to dramatically increase osmolyte biosynthesis in plants are counterproductive, as they simply deplete plant energy resources. This is the most likely reason for why this approach has not resulted in any truly tolerant cultivars in farmers' fields (125).

An outlook: where to from now?

Despite the importance of osmotic adjustment and the role of inorganic ion uptake in this process, specific ionic mechanisms involved are still elusive. Lew (126) had initially suggested that Arabidopsis root hair cells possess an osmosensing but not a turgor-sensing mechanism. However, it was later clearly shown (53) that the root hair turgor is regulated, and this could not happen without turgor sensing. Several papers have argued for the presence of mechanosensitive, or stretch-activated, channels at the plasma membrane (127, 128) which can sense the cell volume changes and trigger fluxes of appropriate ions to counteract them. The exact mechanism of this modulation, however, remains unclear and should be addressed in future studies. Earlier, Palmgren (129) suggested that relaxation of the stretched status of the membrane might directly activate the plasma membrane H^+-ATPase, as the activity of this enzyme is strictly dependent on the lipid environment. It remains to be answered whether the plasma membrane H^+- pump is a primary target (a receptor) of osmotic stress (130), or merely a component of the complex signaling network controlling the activity of the plasma membrane transporters for other ions (131). Supporting evidence for the former hypothesis includes reports of significant osmotic-induced acidification of the bathing medium (131) and direct measurements of net H^+ extrusion (71, 132, 133). Moreover, electrophysiology of characeae suggests that the proton pump in salt tolerant *Lamprothamnium* is activated by both turgor reduction and a salinity increase (134), keeping the membrane potential difference negative. This means that the inward rectifier can be activated to import K^+, and the outward rectifier inactivated. At the same time, in salt sensitive *Chara australis* the proton pump stops within hours of being exposed to 50 mM NaCl. The H^+ (or OH^-) channels open to dissipate the proton gradient, and cells depolarise, losing K^+ through repetitive action potentials and outward rectifier (135, 136), and resulting in the cell's inability to adjust turgor. The proton electrochemical gradient is also necessary for the Na^+/H^+ antiporter to work.

Although compatible solutes have been a hot agenda item for many years, specific mechanisms by which they assist plant adaptation to osmotic stress are only beginning to emerge. Cuin and Shabala (118) presented some evidence for apoplastic mode of action of some of compatible solutes studied, and the crucial role of depolarisation-activated outward-rectifying K^+ channel (KOR) was demonstrated. However, the precise mechanisms by which low [K_m values of around 0.3 mM (119)] concentrations of organic osmolytes control KOR channels activity and K^+ retention remain unanswered. Addressing this issue will be a major challenge, as direct patch-clamp experiments are simply not possible due to the fact that protoplast solutions contain huge quantities of mannitol or sorbitol that are compatible solutes themselves and, hence, may directly affect channel properties. It is also possible that some compatible solutes protect the proton pump in salt tolerant plants. The external Na^+ can be sequestered in the vacuole and utilised in turgor regulation only when the proton pump is fully functional. The most promising way forward may be combining transgenic approaches with *in planta* physiological measurements of plant adaptive responses at various levels of plant structural organization. Similar studies on bacterial cells are also required to confirm the universal strategy of organisms when dealing with hyperosmotic stress.

Another aspect that warrants further efforts is investigation of the interplay between plant hormonal signalling, osmolyte biosynthesis, and signal transduction pathways to downstream ion channels directly involved in osmotic adjustment. An excellent review published recently by Lehmann et al. (137) highlights the causal relationship between regulation of expression of several proline biosynthesis genes, ABA and salicylic acid. Importantly, reactive oxygen species such as NO or H_2O_2 have been shown to act as mediators of ABA signals and affect proline metabolism under stress conditions (138–141). It should be added in this context that, at the same time, H_2O_2 may also directly regulate a population of

K^+ permeable non-selective cation channels in plant roots (59, 142). Therefore, a more specific connection between ion channel activity, osmolyte accumulation, and hormonal and ROS signalling needs to be established.

Highlights

- The bulk of osmotic adjustment in plant and bacterial cells is achieved by increased accumulation of inorganic osmolytes such as K^+, Na^+ and Cl^-. This is applicable to both halophyte and glycophyte species.
- *De novo* synthesis of compatible solutes is an energetically expensive and slow option, and can be used only for the fine adjustment of the cell osmotic potential.
- The most likely role of organic osmolytes in osmotic adjustment is in osmoprotection of key membrane transport proteins and ROS scavenging.
- Of all ionorganic osmolytes, potassium is the most suitable for being accumulated in high quantities without interfering with cell metabolism.
- The specific mechanisms by which compatible solutes regulate activity of ion transporters remain elusive and require more thorough investigation.
- It can be envisaged that plant breeding for osmotic stress tolerance should target both intracellular K^+ retention and Na^+ sequestration traits in combination.
- Creation of transgenic species with increased levels of organic osmolytes by itself is counterproductive due to high yield penalties; all these attempts should be complemented by a concurrent increase in the accumulation of inorganic ions used for osmotic adjustment.

Acknowledgments

This work was supported by the Australian Research Council and Grain Research and Development Corporation grants to S. Shabala.

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Received March 24, 2011; accepted June 29, 2011

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