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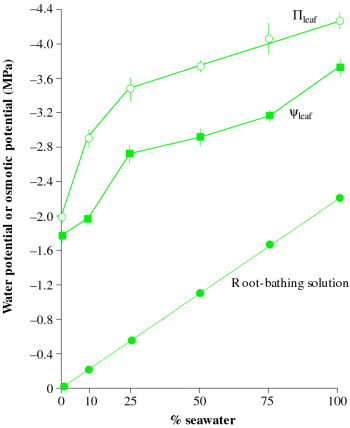
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**Turgor maintenance  17.3.2**

Turgor is crucial to plant function and must be maintained for plants to grow despite salinisation. Reduced water potential in saline soils reduces availability of water to roots, and if trans-piration continues, xylem tension increases and leads eventually to a loss of hydrostatic pressure (turgor) in shoot tissues. Shoot tissues will lose turgor (*P*) unless osmotically active materials accumulate within constituent cells. Accumu-lation of solutes within vacuoles and other osmotic compart-ments raises their osmotic pressure (Π) and water enters via osmosis. If solutes accumulate to an extent that at least matches reduction in bulk tissue water potential (ψ), turgor will be maintained. Such tissue has undergone an ‘osmotic adjustment’.

**Osmotic adjustment  (a)**

Osmotic adjustment is an active process and must be distinguished from a passive increase in solute concentrations due to loss of water during drought or salinisation. An increase in total solute concentration of sap expressed from plant tissues does not constitute osmotic adjustment unless tissue moisture content is taken into account, or more commonly the test material has been rehydrated to full turgor prior to sap extraction. In halophytic plants both soil solutes (inorganic ions) and organic compounds (synthesised *de novo*) can contribute.

[](http://plantsinaction.science.uq.edu.au/edition1/?q=figure_view/855)[1]

**Figure 17.25 Osmotic adjustment in leaves on the mangrove *Avicennia marina* grown in a range of salinities. The decrease in the osmotic potential of the leaf sap matched the decrease in total leaf water potential (mid-morning values), indicating that turgor was maintained. The change in osmotic potential was due largely to accumulation of Na+ and Cl- ions in the leaves. Osmotic potential of root-bathing solution (% seawater) is indicated. (Based on Downton 1982)**

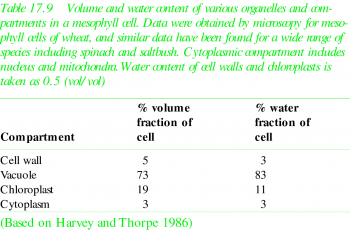
Most plants adjust osmotically to a decline in soil water potential, provided they are allowed sufﬁcient time to synthesise organic compounds and accumulate soil solutes. For example, *Avicennia marina* grown over a large salinity range (Figure 17.25) accumulated leaf solutes according to root-zone salinisation and was able to maintain turgor. In that case (Figure 17.25) osmotic adjustment was largely accomplished by an accumu-lation of Na+ and Cl–. Halophytes commonly accumulate very high concentrations of these ions, despite their potential toxicity. For example, *Atriplex amnicola* growing in a highly saline soil (equivalent to a solution of 400 mM NaCl) tolerated over 900 mM Na+ in mesophyll tissue without visible injury (Aslam *et al*. 1986). Assuming these Na+ ions were balanced by an accumulation of Cl– ions an osmotic adjustment of around 4 MPa must have occurred.

Recognising that enzymes cannot function in salt concen-trations much above 100 mM, most of the salt in leaves of salinised halophytes such as *Atriplex amnicola* is presumably held in vacuoles. Salt concentrations in cytoplasmic compart-ments and cytosol must be kept low enough to allow enzyme function. By implication, salinity tolerance at a cellular level involves vacuolar accumulation of salt, and a balancing accumulation of organic solutes in cytoplasmic compartments as outlined below.

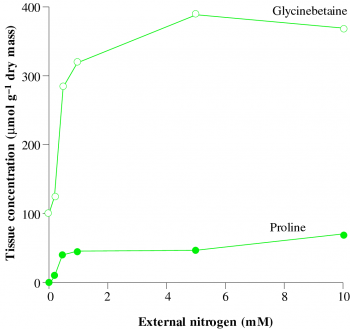
**Compatible solutes  (b)**

Most plants can synthesise and store certain organic molecules without detriment to enzymic activity. Such solutes have been called ‘compatible solutes’, a term coined by A.D. Brown, University of New South Wales, and promoted by Borowitzka and Brown (1974) to describe solutes that were not toxic to metabolism, and that accumulated in unicellular organisms exposed to high salinity. In contrast to compatible solutes, most other metabolites have deleterious effects on enzymes even if their concentrations rise by only a few mM. Criteria for an ideal compatible solute include: low molecular weight to favour high osmotic activity, high solubility in water, electrical neutrality and an absence of metabolic inhibition despite high concentrations. The most outstanding example of a compatible solute in operation is glycerol accumulation in the salt lake alga *Dunaliella parva* (see Case study 17.2).

Several types of compatible solutes are present in halo-phytic vascular plants. These include sugars (e.g. glucose and sucrose), polyhydric alcohols (e.g. mannitol and pinitol), amino acids (e.g. proline and asparagine), betaines (e.g. glycinebetaine and b-alanine betaine), methylated sulphonio compounds (e.g dimethylsulphoniopropionate, DMSP), and some methylated relatives of proline and proline analogues (Naidu *et al*. 1987). Sugars and amino acids are ubiquitous, remaining compounds are not. Compounds such as glycinebetaine accumulate in a diverse range of plant species as well as in bacteria and animals, whereas others such as pinitol are limited to fewer species. The detailed chemistry of methylated nitrogen and sulphur compounds (quaternary ammonium and tertiary sulphonium compounds) falls outside our present scope but has been reviewed by Rhodes and Hanson (1993).

[](http://plantsinaction.science.uq.edu.au/edition1/?q=figure_view/856)[2]

**Table 17.9**

[](http://plantsinaction.science.uq.edu.au/edition1/?q=figure_view/857)[3]

**Figure 17.26 Compatible solutes such as glycinebetaine and proline are nitrogen rich, and their synthesis draws heavily on both energy and nitrogen reserves. A greater external nitrogen supply enhances synthesis of compatible solutes in *Spartina alterniflora* grown at an external salinity of 500 mM NaCl (Based on Colmer *et al.* 1996)**

Some of these organic compounds (osmotica) can be present at very high concentrations in the leaves of halophytes. Proline can represent 10% of leaf dry mass of some halophytes, for example the sedge *Triglochin maritima* (Stewart and Lee 1974). At high salinity *Melaleuca lanceolata* (dryland tea tree) can accumulate methylhydroxyproline to 3.3% of leaf dry mass. In some saltbush species glycinebetaine accumulates in leaves to 5% of dry mass. Organic solutes present at these levels would make a signiﬁcant contribution to osmotic adjustment even if they were not restricted to certain cytoplasmic compart-ments. For example, if proline accounted for 10% of dry mass, that would represent a concentration of 250 mM on a tissue moisture basis (assuming an H2O/DM ratio of 4:1), and would contribute a further 0.6 MPa to the osmotic pressure of that tissue. Recognising that organic osmotica *are* localised in speciﬁc compartments such as chloroplasts, and if such an accumulation was restricted to that compartment, osmotic impact would be boosted 10 times (Table 17.9).

Accumulation of these nitrogen-containing compatible solutes is strongly influenced by nitrogen supply. Proline and glycinebetaine in *Spartina alterniflora* are especially sensitive and increase enormously when total soil nitrogen (nitrate plus ammonium) exceeds 1 mM (Figure 17.26). Salt tolerance will thus depend indirectly on nutrient resources that contribute to synthesis of these nitrogen-rich compatible solutes, and nutrient imbalance brought on by salinisation may well impair full expression of salt tolerance.

**Osmotic adjustment of cell compartments  (c)**

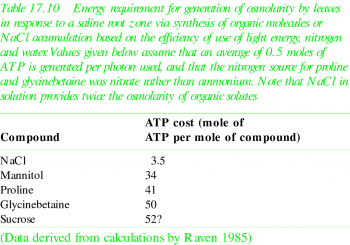
If metabolic function is to withstand salinisation, Na+ and Cl– should be sequestered in cell vacuoles, and organic solutes should accumulate in the cytoplasm and organelles to balance their osmotic effects. However, organelles have a very limited tolerance to changes in volume before metabolic function is perturbed, so osmotic adjustments in cellular compartments must be closely coordinated to preserve cell function.

Volume distribution between various metabolic compart-ments in a typical grass leaf (Table 17.9) shows that a vacuole commonly occupies 50–90% of total cell volume, depending on cell type. Chloroplasts are the next largest fraction, with walls and cytosol constituting a minor fraction by comparison. By contrast, the amount of water per unit volume (osmotic volume) in these various compartments (organelles) is quite different. The water fraction of chloroplasts is only about 0.5, as most of the volume is taken up by thylakoid membranes, starch and soluble proteins. The water fraction of cell walls is also only about 0.5, as most of that volume is occupied by a cellulose matrix. Accordingly, only small amounts of a compatible solute need to be synthesised to achieve an effective osmotic adjustment for cytoplasm and organelles such as chloroplasts, as they occupy only about 15% of the total *osmotic volume* of a mesophyll cell. Such requirements are even less in cell types such as fully expanded root cortex and epidermal cells which have large vacuoles (up to 90% of internal volume) and are equipped with mitochondria and microbodies but lack chloroplasts.

Direct measurement of ion concentration in cell compart-ments is difﬁcult, especially in small compartments that are no more than a few micrometres thick, because ions tend to move this distance during tissue preparation. Ions can be detected by X-ray microanalysis of fresh-frozen tissue, conﬁrming that Na+ and Cl– are preferentially sequestered in the vacuole (see Storey and Walker (1987) for methodology). For example, X-ray microanalysis of tobacco cells growing in 430 mM NaCl indicated that Na+ and Cl– concentrations in vacuoles were about 800 mM and 630 mM respectively, while cytoplasmic concentrations of these ions were near 100 mM (Binzel *et al*. 1988). Kinetic analyses of 22Na+ and 36Cl– efflux from cells conﬁrmed those values.

Direct measurements of organic solutes in various compartments of plant cells are virtually impossible because they cannot yet be measured *in situ*. One way of assessing cellular compartmentation is to isolate organelles from tissues and measure the solutes in the organelle preparations. This approach is not ideal since the organelle contents may leak, and the preparations may not be pure. Nevertheless, data from chloroplast and vacuole isolations generally conﬁrm that organic compounds accumulate in cytoplasmic compartments such as chloroplasts, whereas salts are stored in vacuoles. For example, analyses of vacuole preparations from *Atriplex gmelini* grown at 250 mM NaCl showed glycinebetaine concentrations of 16 mM in the leaf tissue as a whole compared with only 0.2 mM in the vacuole (Matoh *et al*. 1987). Glycinebetaine is not likely to have leaked out of these vacuoles during isolation because they were shown to contain 570 mM Na+ and 260 mM Cl–, which are both reasonable values. In another study (Robinson and Jones 1986) chloroplasts were isolated from spinach (a salt-tolerant plant) grown at 200 mM NaCl. Glycinebetaine concentration in chloroplasts was 290 mM compared with only 15 mM in the leaf as a whole. Glycinebetaine is known to be synthesised in chloroplasts and this study showed that it was the major solute contributing to osmotic adjustment of the chloroplasts when spinach was grown in a saline soil (Robinson and Jones 1986). Such ﬁndings conﬁrm a role for glycinebetaine as a compatible solute that helps preserve cytoplasmic function during salt stress.

**Energetics  (d)**

[](http://plantsinaction.science.uq.edu.au/edition1/?q=figure_view/858)[4]

**Table 17.10**

Saline soil imposes metabolic (energy) costs on halophytes. Those costs include salt exclusion, compartmentation in vacuoles and excretion via salt glands and bladders. Such costs are, however, still small compared to the cost of generating organic solutes for osmotic adjustment (Yeo 1983). Generating enough organic solutes to achieve full osmotic adjustment in a hypersaline soil can consume a large fraction of a plant’s  
available energy. It is much more efﬁcient to use NaCl for osmotic adjustment for two reasons: (1) twice as many organic molecules need to be generated as NaCl molecules accumu-lated, because NaCl dissociates to Na+ and Cl– in solution to almost double its osmotic impact per mole (osmotic coefﬁcients in Table 17.2); (2) ATP requirement for synthesis of an organic molecule is much greater than the number of ATPs required to transport and compartmentalise either an Na+ or Cl– ion in leaves. Approximately four ATP molecules are consumed in using NaCl as an osmoticum in root cells, and seven ATP molecules are needed in leaf cells. By contrast, ATP requirement (mole per mole) for synthesis of organic compounds is an order of magnitude higher (Table 17.10). Halophytes that can compartmentalise high concentrations of salt in vacuoles as an osmoticum are at a strong selective advantage, hence their superior salt tolerance. Additional costs of synthesising organic compounds for much smaller cyto-plasmic compartments then becomes energetically feasible.

As mentioned above, soil nitrogen can limit a plant’s ability to produce nitrogen-containing compatible solutes, and their accumulation may also impose a severe drain on nitrogen metabolism generally. Nitrogen contained in com-pounds such as proline or betaine can represent 10–30% of total shoot nitrogen. For example, in *Spartina alterniflora*, when the leaves accumulated glycinebetaine to 4.4% of their dry weight, this locked up 12.5% of the total leaf nitrogen. More-over, nitrogen and carbon contained in these compounds is unavailable for biosynthetic processes or as a substrate for respiration. Contrary to expectation, plants do not metabolise glycine-betaine. They certainly do metabolise proline, and it is rapidly broken down following stress relief to release nitrogen and reducing power from these nitrogen-rich and energy-rich compounds. No instances are reported of plants breaking down glycinebetaine or any of the methylated amino acids or analogues of proline following stress relief.

Synthesis of nitrogen-rich and energy-intensive organic solutes may well carry a strong selective advantage for vascular plants as a counter to salt stress, but some trade-off against growth is inevitable. By comparison, NaCl is a rather ‘cheap’ solute in metabolic terms, and NaCl-based osmotic adjustment constitutes an important adaptive feature of halophytes.