17.3.1??Devices to manage leaf salt

(a)??Ion exclusion

Halophytes depend heavily on salt exclusion by roots to regulate their internal salt load. Non-halophytes are similarly reliant on exclusion mechanisms (Section 17.2) but halo-phytes cope with much greater salt loads. For example, the salt concentration in xylem sap of the coastal mangrove *Avicennia marina* stays as low as 9 mM despite a sediment concentration of around 500 mM NaCl, that is, 98% exclusion is achieved. This degree of exclusion even exceeds that of barley, one of the most salt tolerant non-halophytes (Figure 17.3; Table 17.3). Exclusion is particularly important for perennial halo-phytes because their leaves live for a year or more and need to regulate incoming salt over a much longer period compared with annual plants where leaves usually live for a month or so.

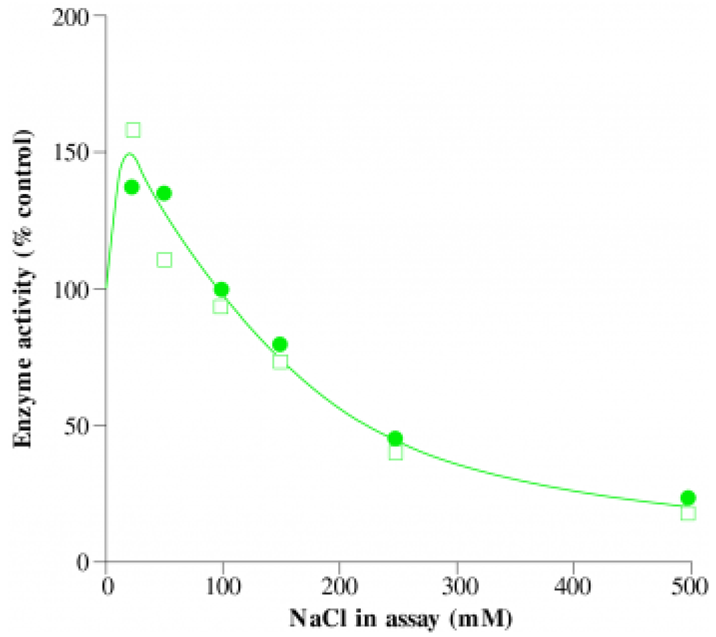

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Figure 17.20 Enzymes from halophytes are not intrinsically salt tolerant. NaCl effects on the activity of malate dehydrogenase from saltbush, *Atriplex spongiosa* (closed symbols), and bean, *Phaseolus vulgaris* (open symbols), confirm comparable sensitivity. A number of enzymes extracted from the halophyte *Suaeda maritima* and pea (*Pisum sativum*) were also equally inhibited by NaCl *in vitro*. (Based on Greenway and Osmond 1972; Flowers 1972)

Regulation of leaf salt is crucial because enzymes of vascular halophytes are just as sensitive to NaCl as enzymes of non-halophytes, but nevertheless operate against a background of higher salinity. For example, enzymes extracted from the halophytes *Atriplex spongiosa* or *Suaeda maritima* were just as sensitive to NaCl in their assay media as were enzymes extracted from bean or green pea (Figure 17.20). Moreover, protein synthesis of an *in vitro* translation system of polysomal preparations from halophytes was also sensitive to salt; trans-lation was completely inhibited by 200 mM NaCl (Flowers and Dalmond 1992). Even enzymes from the pink salt lake algae *Dunaliella parva*, which can grow at salinities 10-fold higher than those of seawater, were found to be sensitive to NaCl (reviewed by Munns *et al*. 1983). Isolated as a cell-free preparation, enzymes from halophytic plants are just as salt sensitive as their counterparts in non-halophytic plants.

(b)??Ion excretion — salt glands and bladders

In addition to effective salt exclusion by roots and vascular systems within shoots, many halophytes carry salt glands or salt bladders that shift ions from mesophyll tissues to leaf surfaces. Here solutes crystallise and are eventually blown or washed away. Salt glands are highly specialised organs which consist of several cells designed to excrete salt; salt bladders are modi?ed epidermal hair cells that accumulate salt and often burst, releasing their contents. Excretion is an energy-dependent transport of ions across membranes against large concentration gradients. Both structures excrete mainly Na^+ and Cl^-, together with lower concentrations of other ions according to soil composition. For example, in *Tamarix aphylla* (native to the desert steppes of Asia Minor and northwest India) salt glands on trees growing on alluvial soils in northwestern Victoria excrete a variety of ions including Ca^{2+}, Mg^{2+} and SO_4^{2-} (Storey and Thomson 1994).

Salt glands occur in a number of halophytic species, including several families of dicotyledons and monocotyledons that are not closely related taxonomically, and thus provide a clear example of convergent evolution on a common adaptive device. Salt bladders, on the other hand, are con?ned to members of the family Chenopodiaceae.

Salt glands

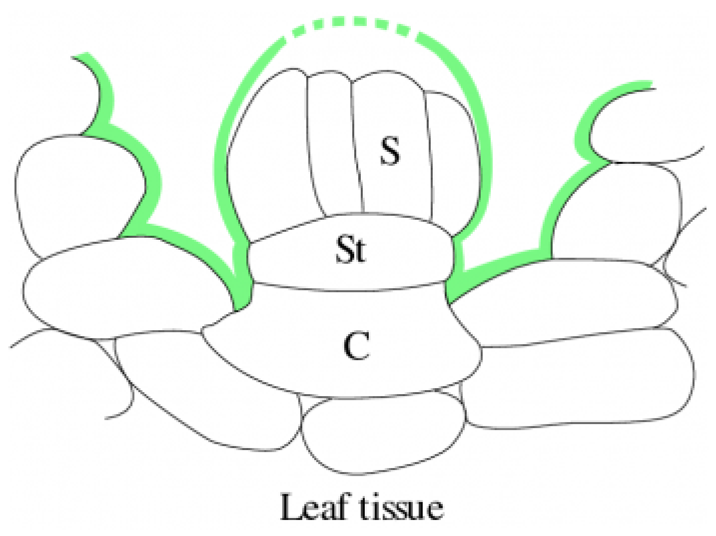

Figure 17.21 Diagram of a salt gland from the mangrove *Avicennia marian*, showing a collective cell, C (a modified epidermal cell), a stalk cell, St, and secretory cells, S. A cuticle (shaded layer) extends over the gland and is perforated above the secretory cells to allow excretion of a concentrated salt solution. (Based on original transmission electron micrograph by Jocelyn L. Carpenter, Biological Sciences, University o Sydney; see also Carpenter *et al.* 1990)

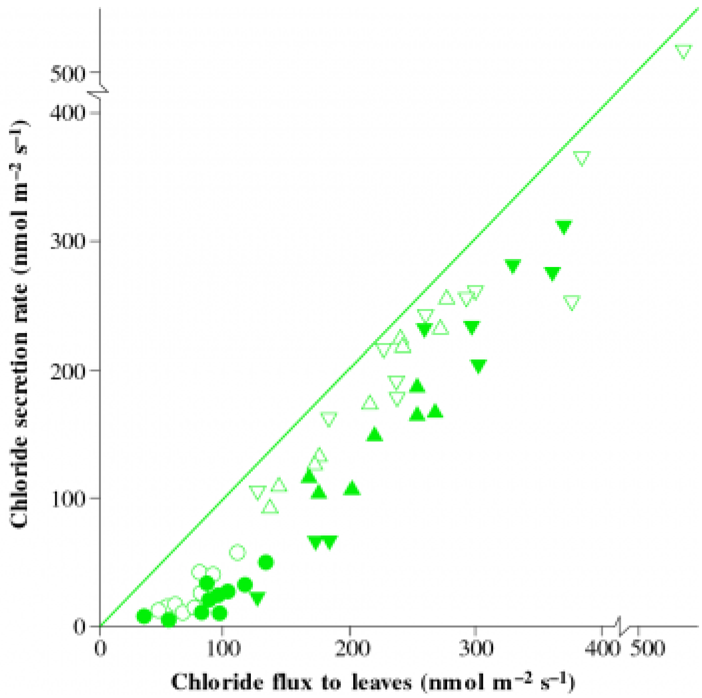

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Figure 17.22 Excretion rates from mature leaves of two mangroves, *Avicennia marina* (solid symbols) and *Aegicerus corniculatum* (open symbols), growing at various salinities correlate closely with xylem flux of Cl^- ions to those leaves; circles = 50 mM NaCl; triangles = 250 mM NaCl; inverted triangles = 500 mM NaCl. The solid line through the origin represents a hypothetical case where all salt is excreted and none accumulated. Salt excretion represents an important component of salt tolerance in halophytes and emphasises the remarkable capacity of salt glands (Based on Ball 1988)

Salt glands are made up of several specialised cells, located in slight depressions of leaf epiderms and covered by the cuticle. Structural details vary according to species. In *Avicennia marina* (Figure 17.21) there are two to four collecting cells that are adjacent to the mesophyll, a stalk cell and 8–12 secretory cells that discharge the salt through pores in the cuticle. Excretion requires energy and drops to very low rates during darkness.

When plants are growing in seawater, ions excreted are predominantly Na^+ and Cl^-, and rate of excretion increases with increasing salinity (Figure 17.22). Salt excretion via glands removes a signi?cant proportion of the total salt transported to mangrove leaves. In *Avicennia marina*, as much as half the salt reaching the leaves may be excreted, and the salt glands of other mangroves such as *Aegiceras corniculatum* excrete an even higher proportion of salt reaching their leaves (up to 90% at high salinities; Figure 17.22). Clearly, salt glands are signi?cant devices for management of salt loads of such halophytes.

Salt glands of halophytic monocotyledons are structurally simpler than those of dicotyledons and usually consist of just two cells. Nevertheless, these simpli?ed glands are still capable of high rates of excretion. Salt-tolerant Kallar grass *Diplachne fusca* (Figure 17.18) excretes salt at rates comparable to mangrove.

Salt bladders

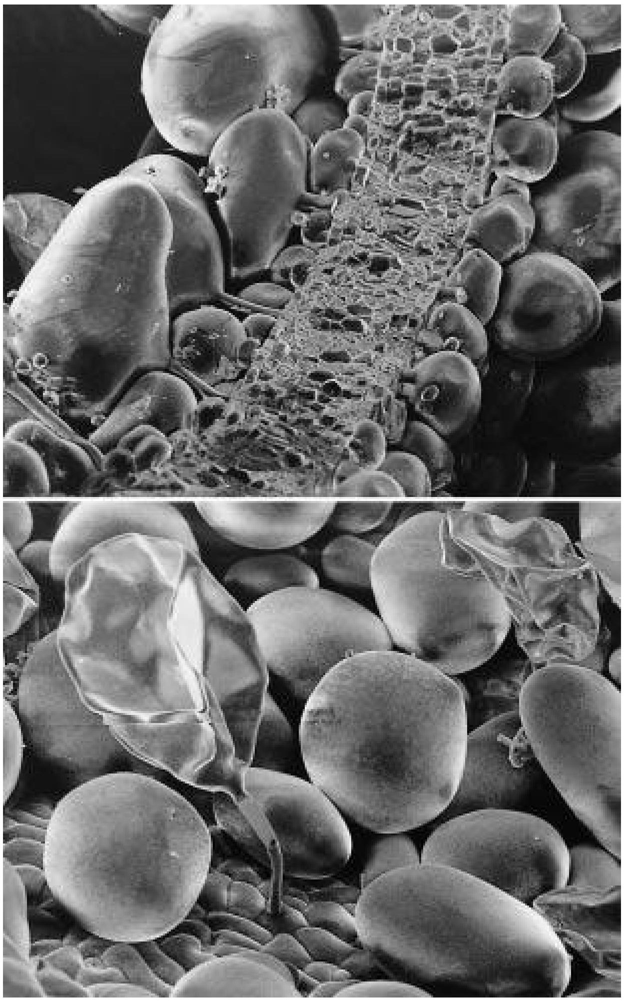
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Figure 17.23 Scanning electron micrographs of a transverse section through a young leaf (a) and a surface view of a mature leaf (b) of *Atriplex semilunaris*. Leaves on many *Atriplex* species are covered by trichomes (enlarged into salt bladders) that form a dense and water-repellent silvery layer on both surfaces. More than 50% of salt entering leaves can be subsequently secreted into these bladders by their intensely cytoplasmic stalk cells. Bladders swell during leaf expansion

and eventually rupture to release their contents, revealing extensive elongation of stalk cells in this particular species. SB = swollen bladder, CB = collapsed bladder, E = epidermis, M = mesophyll and S = stalk. Scale bars = 50 µm (Scanning electron micrographs courtesy Richard Storey)

Development of salt bladders is particularly notable on the more salt tolerant members of the family Chenopodiaceae, which includes saltbushes (*Atriplex* sp.). Salt bladders are modi?ed epidermal hairs, and usually consist of two cells, a stalk cell and a bladder cell (Figure 17.23a, b). Stalk cells transport ions from mesophyll cells to bladder cells, where NaCl builds up to extremely high concentrations. Bladder cells are covered by a cuticle which is impermeable to both water and salt. As salts are accumulated by bladder cells they expand enormously, and may reach a ?nal diameter of up to 200 µm. Eventually a bladder cell bursts (Figure 17.23b), discharging salt onto leaf surfaces.

Accumulation of salt in bladders prevents excessive build up in mesophyll cells. Such protection is particularly important for young leaves. In many *Atriplex* species, bladder cells sequester the bulk of salt reaching young leaves, and concentrations in bladder cells can be up to ?ve times higher than those in mesophyll cells. Taking into account the relative volumes of the bladders versus the rest of the leaf, such bladders hold over 80% of the total leaf Na^+ of young leaves. However, as leaves age, the amount of Na^+ stored in bladders does not continue to increase, and is eventually matched by levels in mesophyll tissue.

(c)??Succulence

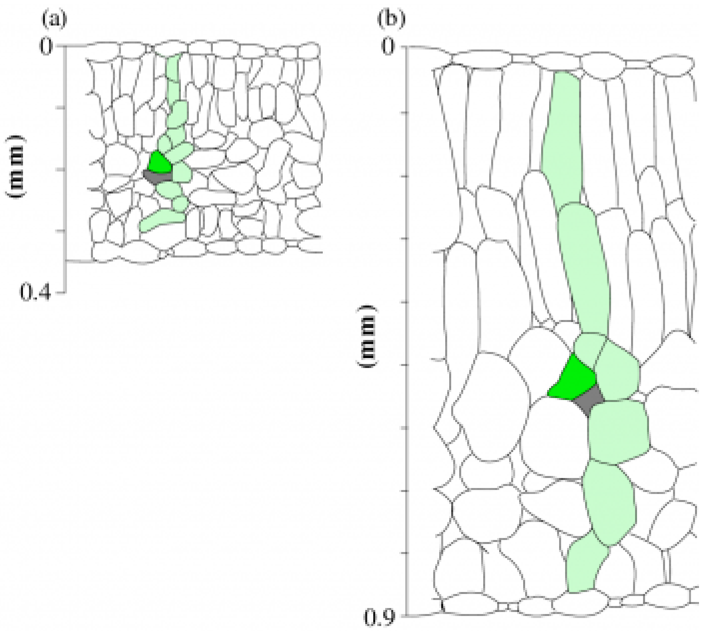

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Figure 17.24 NaCl has a spectacular effect on leaf succulence in mature leaves of *Atriplex hastata*. Plants were grown either without NaCl (a) or with 600 mM NaCl (b). Xylem is shown in dark green, phloem is grey. Light green bands identify corresponding cells across these transverse sections and confirm that salt-induced succulence involves an increase in cell volume (hence thicker leaves) rather than cell number. The scales show leaf thickness (Redrawn from Black 1958)

Thick or fleshy leaves contain large and highly vacuolated cells (Figure 17.24). Leaf succulence is then measured as the maximum water content (expressed as mass) per unit of leaf area. Succulence is a feature of many dicotyledonous halo-phytes, and is due to an increase in length of palisade cells, an increase in diameter of spongy mesophyll cells, and sometimes the development of an extra layer of cells. How such profound changes in leaf anatomy are instigated remains unknown, but Na^+ ions are speci?cally implicated.

Succulence, as a response to salinisation, can be spectacular and leaves of *Atriplex* spp. usually double in thickness when grown at high salinities. For example, leaves of *A. hastata* increased from 0.4 mm to 0.9 mm when salinity was increased to 100 mM NaCl (Figure 17.24). Larger cells obviously store more salt per unit of transpiring surface area, and leaf thickness continues to increase long after leaves have reached maximum area. As mentioned above, salt bladders are already full by the time leaves reach maximum area, so that succulence offers additional storage of incoming salts, thus alleviating impact on cytoplasmic compartments.

Succulence is not restricted to halophytes. Dicotyledonous plants commonly develop succulence in response to NaCl. Cotton leaves, for example, increase succulence by 50% (from 23 to 36 mg H_2O cm $^{-2}$ leaf area) when grown at 300 mM NaCl. Despite that response, growth was still severely reduced (Longstreth and Nobel 1979). Curiously, succulence has not been reported for monocotyledons.
