Sporobolus virginicus leaf salt glands: morphology and ultrastructure

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The leaves of the halophytic, C_4 grass, *Sporobolus virginicus*, possess bicellular salt glands that are more abundant on the adaxial surface. Each gland is composed of a voluminous, flask-shaped basal cell, embedded in the leaf and a smaller, dome-shaped cap cell that protrudes beyond the epidermis. An ornamented, porose cuticle overlies the protruding gland. The dense cytoplasm of the basal cell is partitioned into channels by a system of paired membranes which originate from invaginations of the plasma membrane, adjacent to the common wall between basal and cap cells. Closely associated with the partitioning membranes are numerous mitochondria, microtubules and endoplasmic reticulum. The basal cell is intimately connected to adjacent cap, epidermal and mesophyll cells by numerous plasmodesmata. The dense cytoplasm of the cap cell lacks partitioning membranes, but contains numerous small vacuoles and a concentration of organelles in close proximity to the outer surface. The basal cell appears to be suitably located and designed to access and direct ions from surrounding mesophyll and epidermal cells into the channels formed by the partitioning membranes. Subsequent ion movement is probably symplastic via the cytoplasm and plasmodesmata to the cap cell. The abundant mitochondria, which are closely associated with the partitioning membranes, are probably important in ion transport through the cytoplasm of the basal cell. The ions appear to be compartmentalised and transported across the cap cell in small vacuoles and accumulate in the cuticular cavity prior to elimination via cuticular pores or through rupture of the cuticle.

Keywords: halophyte, microhairs, partitioning membranes, salt glands, *Sporobolus virginicus*, ultrastructure.

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Introduction

Salt secretion in halophytes is regarded as an adaptive mechanism to regulate the levels of salt in the leaves and to cope with high substrate salinity (Waisel 1972; Naidoo & Naidoo 1998). In the middle of the nineteenth century, salt glands were regarded as special hydathodes or even as chalk glands if Ca^{2+} and Mg^{2+} carbonates or bicarbonates were secreted. Salt glands are specialised epidermal cells that are actively involved in the elimination of mineral elements.

Salt secretory structures are observed in a great variety of halophytes and are common in many families of angiosperms. Three types of salt glands are recognised on the basis of their structural organisation (Thomson 1975): the two-celled glands of the grasses, the bladder cells of the Chenopodiaceae, Mesembryanthemaceae and Oxalidaceae, and the multicellular glands which occur in other dicotyledonous families. Salt glands may be simple or complex consisting of between 2–40 cells (Lüttge 1971). In the Poaceae, glandular structures may be either bicellular or multicellular (Linder *et al.* 1990). The 2-celled structures in the grasses are referred to as bicellular trichomes, microhairs or salt glands. The salt glands of grasses are a feature of the lower and upper epidermis of leaf blades, lemmas, paleas and lodicules (Terell & Wergin 1981). Multicellular glands are rare in the Poaceae (Johnston & Watson 1976) and their occurrence and distribution have not been adequately investigated.

Since the first salt glands were described in the Tamaricaceae in the nineteenth century, increasing attention has been given, not only to the structure and function of salt glands, but also to the mechanism of salt secretion and to its ecological significance. While the salt glands of dicotyledons have been investigated extensively (Hill & Hill 1976), detailed studies on monocotyledons were primarily on *Spartina* species (Skelding & Winterbotham 1939; Levering & Thomson 1971). More recent ultrastructural studies on salt glands of grasses include those on *Spartina anglica* (Zhou *et al.* 1982), *Cynodon* and *Distichlis* (Oross & Thomson 1982 a,b) and Amarasinghe and Watson (1988) compared the microhairs of seventeen species of Poaceae from four subfamilies. The nature of the secretion by grass microhairs was investigated using cytochemical techniques (Amarasinghe 1990).

Sporobolus virginicus L. (Kunth), which belongs to the subfamily Chloridoideae (Poaceae), is a halophytic, mat-forming, stoloniferous, perennial grass that is widely distributed in South Africa along the coastal areas of the Cape and KwaZulu-Natal (Gibbs Russell *et al.* 1990). It is an important pioneer species under saline conditions along coastal lagoons, estuaries, bays and sandy beaches. Although common along the coast, this species also occurs inland around saline water bodies. The leaves of *S. virginicus* possess salt glands predominantly on their adaxial surfaces. The aim of this study was to determine the structure of the salt glands of *S. virginicus* at the light and electron microscope levels and to relate these to function. Ion transport pathways and the ecophysiology of salt secretion are addressed in subsequent papers (Naidoo & Naidoo 1998).

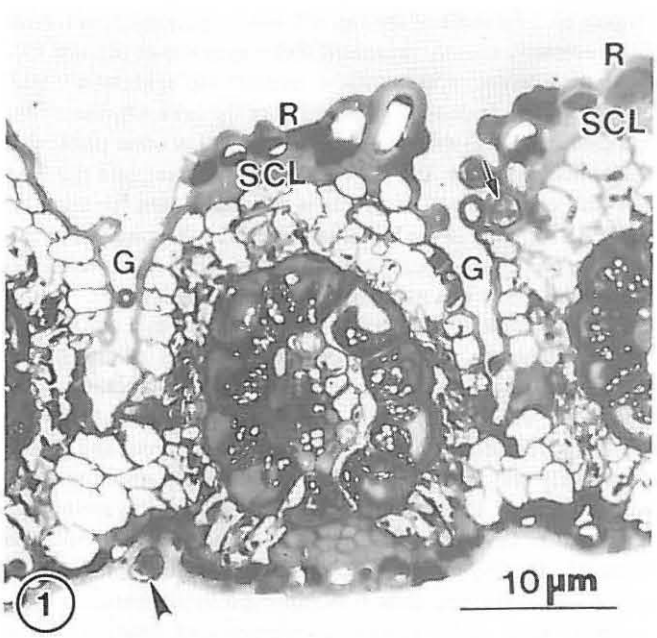
Materials and Methods

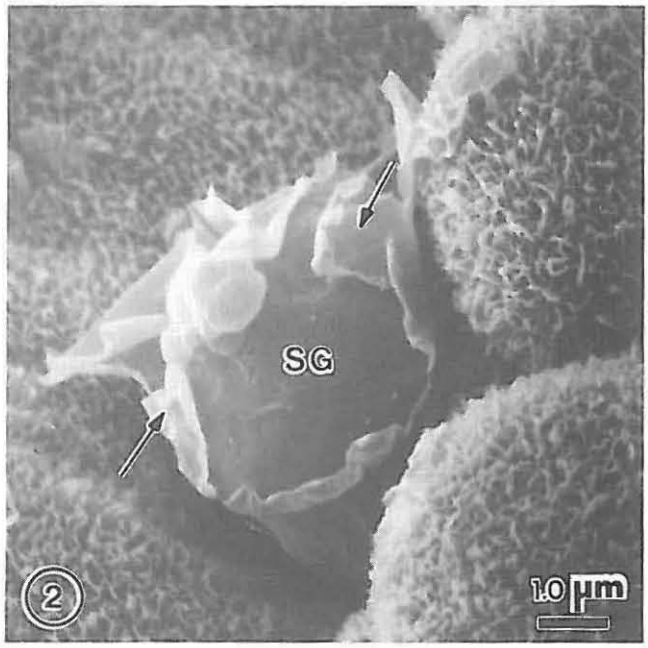
Plant material

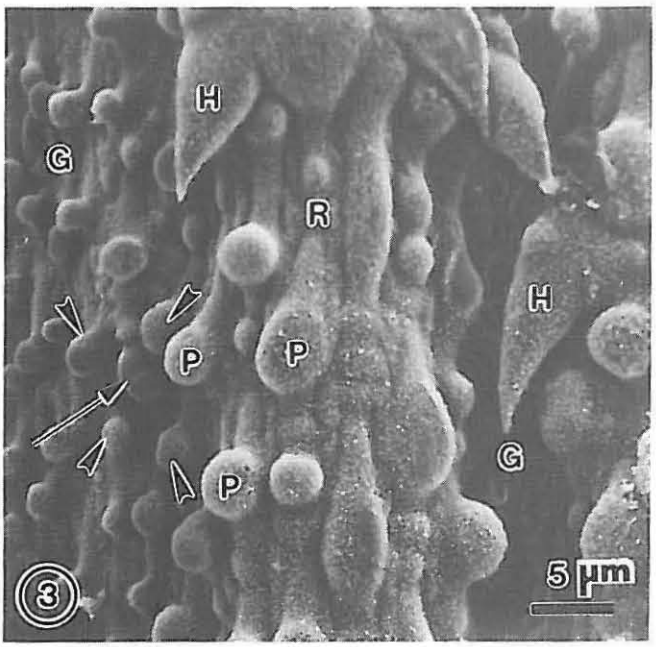
Terminal rhizomes of *S. virginicus* were collected from the Beachwood Mangroves Nature Reserve, Durban, KwaZulu-Natal. For light and electron microscopy samples were taken from the mid-region of flag leaves (i.e. the last fully expanded leaves).

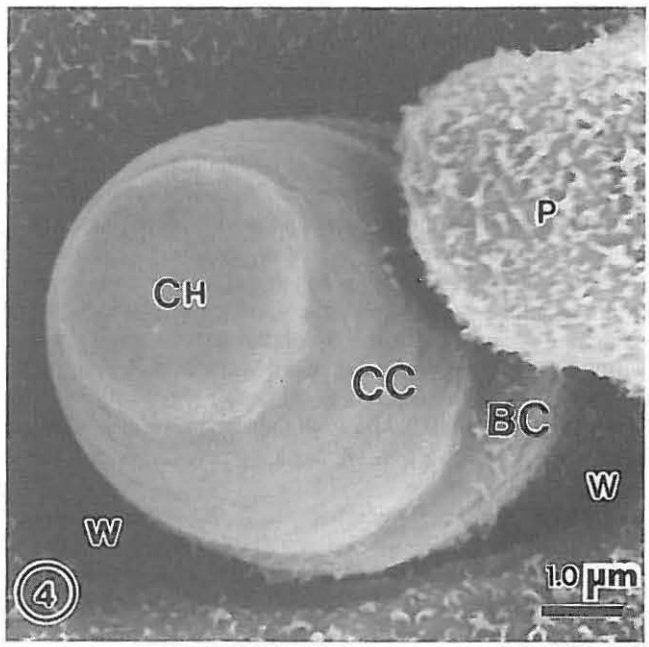
TEM (Transmission electron microscopy)

Small segments of leaf tissue (1 mm^2) were fixed in cold 3% glutaraldehyde (4°C) buffered with 0.1 M sodium cacodylate (pH 7.2) for approximately 4–6 h. The samples were then post-fixed in 1% aqueous osmium tetroxide (O_sO_4) for 4 h at room temperature in a dark cupboard, dehydrated through a graded series of ethanol and embedded in 100% Spurr's (1969) low viscosity resin. Ultrathin sections were cut using conventional methods and post stained with 2 % aqueous uranyl acetate followed by Reynold's (1963) lead citrate. Sections were observed and photographed with a Philips TEM 301 operated at 60 or 80 kV.









Figures 1–4 **(1)** Toluidine blue stained section of leaf showing adaxial gland (arrow) inserted under sclerenchyma cap (SCL) and abaxial gland (arrowhead). Note undulating leaf surface with ridges (R) and grooves (G). **(2)** Ruptured cuticle (arrows) over salt gland (SG). **(3)** Part of adaxial leaf surface showing ridges (R), grooves (G), papillae (P) and prickle hairs (H). Note salt gland (arrow) located along lateral wall of groove and partially surrounded by four papillae (arrowheads). **(4)** Bicellular salt gland of *S. virginicus* comprising flask-shaped basal cell (BC) and dome-shaped cap cell (CC) and raised cuticular chamber (CH). Note area termed 'well' (W) around gland and papillae (P) partially surrounding gland.

SEM (Scanning electron microscopy)

Two methods of sample preparation were used for SEM i.e. chemical fixation and freeze-drying. For chemical fixation, material was fixed, post-fixed and dehydrated as described for TEM, critical-point dried, sputter-coated with gold and observed with a Philips SEM 500 operated at 12 kV. For freeze-drying, pieces of leaf blade material were rapidly quenched in liquid nitrogen and then freeze-dried in an Edwards Modulyo freeze dryer at -40° to -60°C at a vacuum of 10^{-2} Torr for 72 h. Leaf segments, secured onto brass stubs with carbon conductive tape, were coated with gold and viewed in a Philips SEM 500 at 12 kV with a working distance of 15 mm.

The salt secretions on freeze-dried leaf surfaces were analysed by energy dispersive X-ray microanalysis (EDX), using the Noran 'Voyager' 2100 EDX system interfaced with a Jeol SEM 6100 operating at 15 kV with a working distance of 12 mm. Spot analyses (1 µm) were performed on individual crystals.

Light microscopy

Semi-thin sections of leaf blade material, embedded either in Spurr's (1969) resin or in LKB-Historesin, were used for light microscopy. An LKB-Historesin embedding kit was used for embedding material in glycol methacrylate, following procedures specified by the manufacturers. Sections of resin embedded material were stained with 0.5% Toluidine blue-O (Feder & O'Brien 1968). Colour photographs were taken with a Zeiss Axiophot Photomicroscope.

Histochemical tests on historesin embedded sections were used for identification of some of the chemical constituents in the leaf cells. Sudan black B was used to determine the presence of suberin

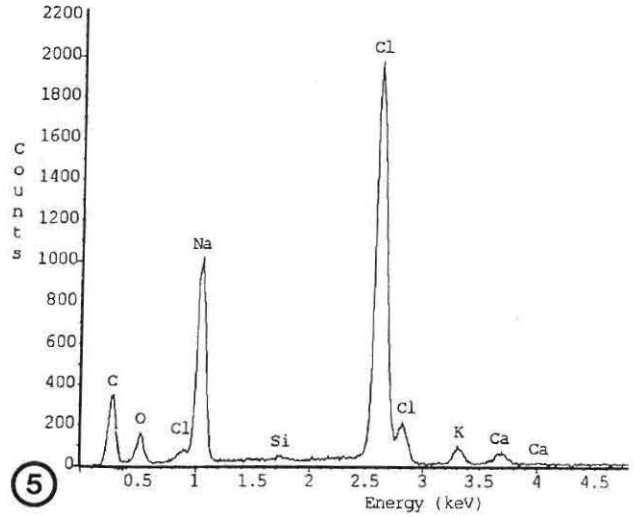


Figure 5 Spot analysis: Spectrum showing the elemental composition of crystalline deposits associated with salt glands of *S. virginicus*.

and Sudan III and IV for cutin (O'Brien & McCully 1981). Transverse sections of fresh leaves were treated with ruthenium red (Jensen 1962) for the detection of pectin in the cell walls.

Results

The leaf surface of *S. virginicus* is undulating with tall adaxial ridges alternating with deep grooves or furrows (Figure 1). On the ridges are numerous papillae and prickle hairs (Figure 3), as well as secreted salts which appear as crystals. Salt glands in *S. virginicus* are globose or club-shaped and readily distinguishable from unicellular papillae and sharp-pointed prickles (Figure 3). Salt glands occur predominantly on the adaxial surface and are uniformly localised along the lateral walls of the grooves, just below the sclerenchymatous cap (Figures 1 and 3). A single row of glands occurs in the grooves of the abaxial surface while two longitudinal rows of glands are located on the flanks of each vein on the adaxial surface.

The bicellular glands comprise a large, flask-shaped basal cell and a small, dome-shaped cap cell (Figure 4). The glands are protected in the groove by four epidermal trichomes (Figure 3) which comprise the salt gland complex. Between the gland and the trichomes, where salt accumulates, is a well (Figure 4). Salts secreted by glands crystallise in the grooves. Crystalline deposits were more abundant on the adaxial leaf surface because of the higher gland frequency. The prismatic crystals over the glands, were primarily Na^+ and Cl^- (Figure 5) although other ions such as K^+ and Ca^{2+} were also detected.

The voluminous basal cell, also referred to as the collecting or stalk cell, is large and embedded in the leaf surface and contiguous with cells of the epidermis and mesophyll (Figure 6). The upper portion of the basal cell is constricted and shaped like a bottle-neck. The secretory or cap cell arises from the neck-like extension of the basal cell. The neck of the basal cell and the cap cell protrude from the leaf surface and are surrounded closely by adjacent epidermal trichomes (Figure 6). Basal and cap cells have walls that are typically 0.5–0.8 µm thick. The walls of the basal cell have a striated appearance and appear to be composed of loose fibrillar material. The thick lateral walls of the bottle-neck region of the basal cell are heavily suberised (results not presented). Treatment of cross-sections of fresh leaves with phloroglucinol indicated no lignification in this region (results not presented). An intermixing of the cuticle, basal cell wall and adjacent epidermal cell wall occurs towards the base of the well (Figure 6). The walls of the cap cell have a pectinaceous (results not presented), loosely organised, flaky appearance (Figure 13).

An ornamented, porose cuticle, overlies the epidermis (Figure 6). The cuticle is distinctly thicker over the area adjoining basal and epidermal cells than that over the cap cell or other parts of the epidermis. However, the cuticle does not impregnate the basal cell wall completely. The cuticle is separated from the outer cap cell wall, resulting in the formation of a salt collecting chamber or cuticular cavity (Figures 6 and 14). However, over most of the glands the cuticle was not intact, but ruptured (Figure 2).

The most striking and unique feature of the basal cell is a system of numerous and extensive invaginations of the plasma membrane, called 'partitioning' or paired membranes which extend into the basal cell from the wall between the basal and the cap cells (Figures 6 and 7). These invaginations anastomose extensively and form a network of tubules deep inside the basal cell (Figure 8). The closed ends of the partitioning membranes terminate in the basal cell cytoplasm (Figure 6). The invaginations result in the formation of membrane-bound channels (Figures 6 and 7) that are open to the apoplast in the direction of the secretory flow. At the point of convergence of several partitioning membrane profiles, there is a unique lattice-like configuration of the membranes (Figure 9). The numerous vesicles in the membrane-bound channels represent cross-sectional profiles of

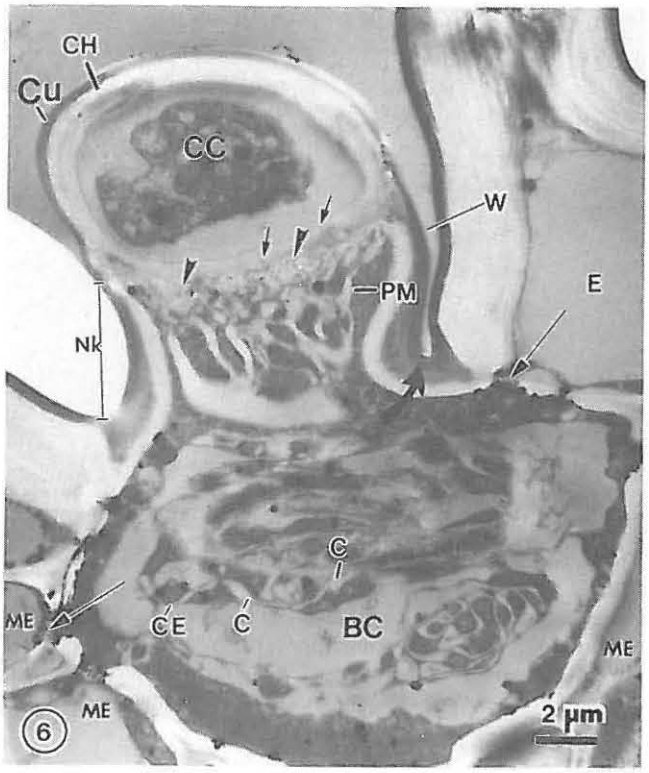
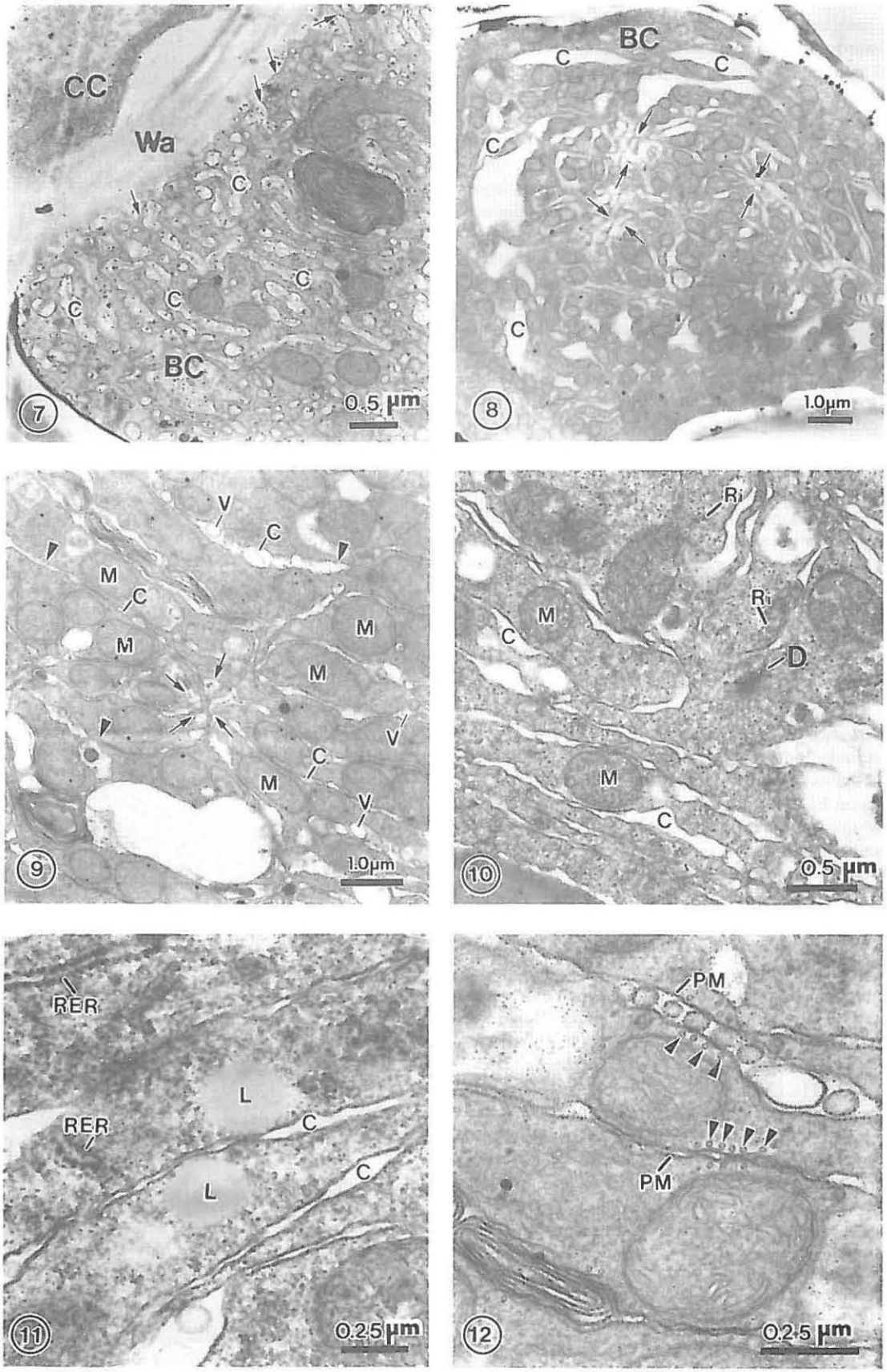


Figure 6 Longitudinal section of bicellular salt gland with flask-shaped basal cell (BC) and dome-shaped cap cell (CC). Continuous cuticle (Cu) covers the surface and well region of the gland. Note separation of cuticle from outer cap cell wall, forming a collecting chamber or cuticular cavity (CH). An intermixing of cuticle, basal cell wall and adjacent epidermal cell wall occurs towards the base of the well (curved arrow). Partitioning membranes (arrowheads) originate at common wall (open ends) between basal and cap cells and terminate in basal cell cytoplasm (closed ends, CE). Note membrane-bound channels (C), thick suberised walls of neck region (Nk) of basal cell, adjacent epidermal trichomes (E) and plasmodesmatal connections between cap and basal cells (short arrows) and between basal and adjacent epidermal (E) and mesophyll cells (ME) (long arrows).



Figures 7–12 **(7)** L.S. basal cell showing origin of membrane-bound channels (C) from plasma membrane invaginations (arrows) at common wall (Wa) between cap (CC) and basal cells (BC). **(8)** T.S. basal cell showing extensive anastomoses of membrane-bound channels (C) forming a tubular network (arrows). **(9)** Part of basal cell cytoplasm showing congregation of channels forming a lattice-like structure (arrows), and numerous mitochondria (M) along membrane-bound channels (C). The numerous vesicles (V) in membrane bound channels represent cross-sectional profiles of plasma membrane invaginations. **(10)** Dense cytoplasm of basal cell with ribosomes (Ri), scattered dictyosomes (D), and mitochondria (M). (11) Segments of rough endoplasmic reticulum (RER) and lipid bodies (L) interspersed between membrane-bound channels (C). **(12)** Numerous microtubules (arrowheads) closely aligned to intracellular face of partitioning membranes (PM).

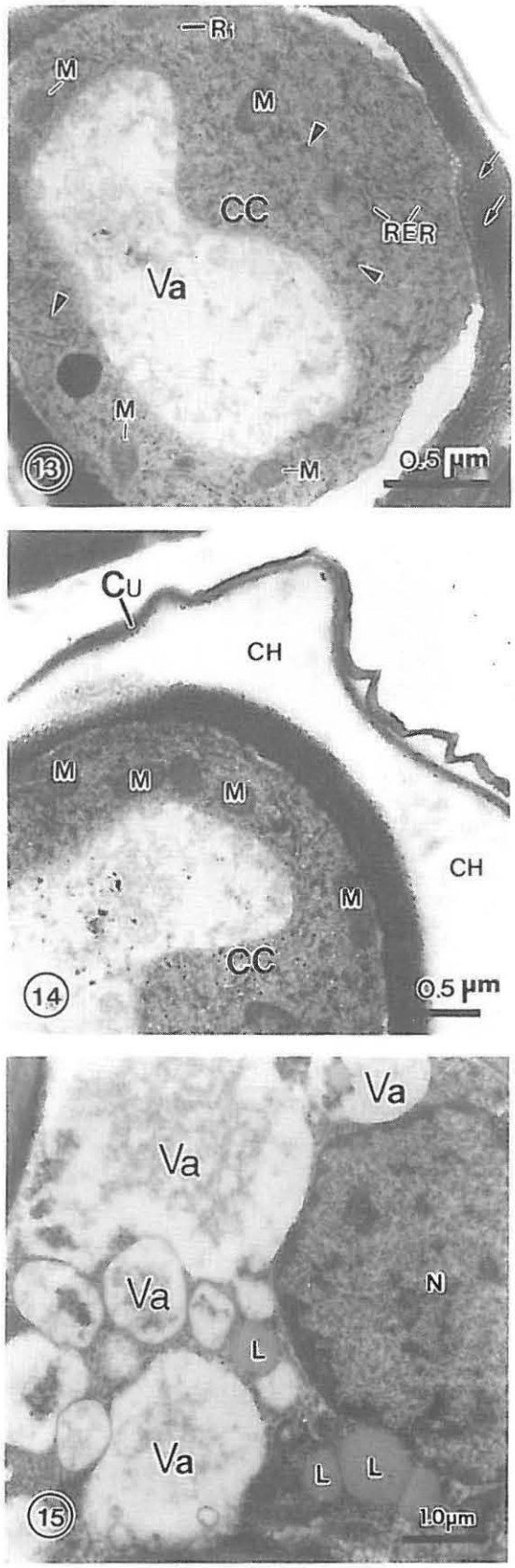
the plasma membrane invaginations (Figure 9). Longitudinal sections of the basal cell indicate that the partitioning membranes are aligned towards the common wall separating the cap and basal cells and are continuous with the plasmalemma along this wall. The basal cell has relatively dense cytoplasm with a large basally located nucleus. Present in the cytoplasm, between the partitioning membranes, are segments of rough endoplasmic reticulum, dictyosomes, small vacuoles, lipid bodies, rudimentary plastids, polysomes and free ribosomes and numerous large mitochondria (Figures 10 and 11). The mitochondria are aligned in the cytoplasmic fingers between the partitioning membranes (Figure 9). Of particular interest is the close association of many microtubules parallel to the intracellular face of the partitioning membranes (Figure 12). Numerous plasmodesmata interconnect the basal and cap cells and the basal and adjacent mesophyll and epidermal cells (Figure 6).

The cap cell lacks partitioning membranes (Figure 13) but is distinguished by the presence of an expanded cuticle which ensheaths the external surface of the gland (Figure 14). The cap cell shows little structural specialisation compared to the basal cell and contains a large nucleus, a few rudimentary plastids, lipid bodies (Figures 13 and 15), primarily rough endoplasmic reticulum cisternae and dictyosomes (Figures 13 and 15). The cap cell is characterised by numerous small vacuoles that contain membranous structures and amorphous material (Figure 15). Microtubules and vesicles are closely associated with the plasma membrane. The cytoplasm of the cap cell is dense, consisting of ribosomes and polysomes (Figure 13). Mitochondria are common, but less numerous than in the basal cell (Figures 13 and 14).

Discussion

Light and SEM indicated conclusively that salt glands in *S. virginicus* are distributed on both leaf surfaces and not restricted to the adaxial surface as reported earlier (Amarasinghe & Watson 1989). The data on EDX micro-analysis verified that the glands are the specific sites of secretion and that the ionic composition is predominantly Na^+ and Cl^-, as reported for other grasses such as *Chloris gayana, Cynodon dactylon, Leptochloa digitata* and *Zoysia macrantha* (Amarasinghe & Watson 1989). Salt secretion in *S. virginicus* was shown to be effective in maintaining salt balance at low to moderate salinities and ineffective at high salinities. High rates of Na^+ secretion and greater retention of K^+ at high salinities maintained stable Na^+/K^+ ratios which may determine the activity of key enzymes such as ATPase (Naidoo & Naidoo 1998). The salt glands of *S. virginicus* have the same general features as those of other grasses such as *Spartina* (Levering & Thomson 1971), *Cynodon, Distichlis* (Oross & Thomson 1982 a,b), in being simple and bicellular. Salt glands vary somewhat in different genera in that they may be sunken as in *Spartina, Sporobolus,* semi-sunken as in *Tetrapogon* or located above the epidermis as in *Bouteloua* (Liphschitz *et al.* 1974; Liphschitz & Waisel 1974, 1982; Naidoo 1990).

Within the Poaceae, slight variations in morphology of the basal and cap cells appear to be related to the efficiency of salt secretion (Thomson *et al.* 1988). Species like *S. virginicus, Spartina* (Levering & Thomson 1971), *Cynodon* and *Distichlis* (Oross & Thomson 1982a) with large flask-shaped, sunken basal cells and dome-shaped cap cells secrete salts more efficiently than trichome-like glands with narrow basal and cap cells as in *Bouteloua* (Liphschitz & Waisel 1974) and *Sorghum halepense* (McWhorter *et al.* 1995). In *S. virginicus* the large sunken basal cell, in close proximity to the veins and adjacent mesophyll cells (Figure 1) is well located to access and channel ions entering the leaf towards the cap cell. Symplastic continuity indicated by numerous plasmodesmata between gland and adjacent cells probably facilitates rapid and selective movement of ions from



Figures 13–15 **(13)** Dense cytoplasm of cap cell (CC) due to numerous free ribosomes (Ri) and polysomes (arrowheads). Note fewer mitochondria (M) than basal cell and abundant cisternae of RER. Cap cell cytoplasm not partitioned by a system of membranes. **(14)** Part of cap cell showing expanded cuticle (Cu) and cuticular chamber (CH) over outer dome of cap cell (CC). **(15)** Large nucleus (N), lipid bodies (L) and numerous small vacuoles (Va) in cytoplasm of cap cell.

the leaf veins to the exterior.

The lateral walls in the neck region of the basal cell, however, are suberised (results not presented) thereby ensuring selective symplastic transport of ions between the basal and cap cells. Lignified lateral walls in basal cells have been documented in *Spartina, Chloris* and *Cynodon* (Liphschitz & Waisel 1974; Oross & Thomson 1982b). The two most prominent ultrastructural features of the basal cell of *S. virginicus* are the numerous membranes which partition the basal cell cytoplasm and the large mitochondria with which they are closely associated (Naidoo *et al.* 1992). Partitioning membranes of the basal cells of salt glands of *Sporobolus, Spartina, Cynodon* and *Distichlis* represent highly unusual subcellular structures (Amarsinghe & Watson 1988) that are adapted for salt secretion (Oross *et al.* 1985). A similar system of membranes is known to occur only in the nectariferous glands of *Asclepias curassavica* (Schnepf & Christ 1980). These partitioning membranes are extensive invaginations of the plasma membrane, with the space between them probably serving as extracellular channels. The geometry of the extracellular channels appears to be consistent in gland cells of grasses (Levering & Thomson 1971; Oross & Thomson 1982 a,b). These channels are open to the apoplast at the common wall separating the basal and cap cell and are closed at the opposite end (Figure 6). The channels are, therefore, open in the direction of the secretory flow and appear to be intimately involved in the secretory process (Levering & Thomson 1971). Movement of water into the lumina of the partitioning membranes occurs when solutes are actively loaded into the extracellular channels (Diamond & Bossert 1967). The standing osmotic gradient, so established, could then effect the movement of salts into the cytoplasm towards the cap cell and eventual secretion by diffusion and mass flow. Rod-like cell wall protuberances and infoldings of the plasma membrane reported in *Spartina* (Levering & Thomson 1971) were not observed either in the basal cells of *S. virginicus* or in *Cynodon* and *Distichlis* (Oross & Thomson 1982a).

The basal cell of the gland of *S. virginicus*, like that of *Cynodon* and *Distichlis* (Oross & Thomson 1982a,b), is analogous to a transfer cell, characterised by numerous plasma membrane invaginations that amplify surface area, thereby facilitating the rate of solute exchange between the apoplast and symplast (Gunn ng & Pate 1969; Oross & Thomson 1982b, 1984). Increased plasma membrane surface through labyrinthine arrays of protuberances also occurs along the walls of the secretory cells of some multicellular glands such as *Tamarix, Frankenia, Limonium* (Ziegler & Luttge 1966; Thomson & Liu 1967) and *Glaux* (Rozema *et al.* 1977).

The lattice-like configuration of the ends of the partitioning membranes in *S. virginicus*, as well as in *Spartina, Cynodon* and *Distichlis*, may represent a 'reservoir' of membranes which expand when secretion is at its maximum (Levering & Thomson 1971). The close association between microtubules and partitioning membranes in *S. virginicus* was also reported in salt glands of *Cynodon* and *Distichlis* (Oross & Thomson 1982a) and in several chloridoid type microhairs (Amarasinghe & Watson 1988). The microtubules were orientated parallel to the secretory pathway suggesting that they may be involved in the secretion process or that they may provide a cytoskeletal support system for the partitioning membranes (Oross & Thomson 1982a; Amarasinghe & Watson 1988). The structural similarity of the partitioning membranes in the glands of *S. virginicus* to those of *Spartina, Cynodon* and *Distichlis* suggests that the secretory mechanism is probably similar in all four grasses. The numerous large mitochondria, closely juxtaposed to the partitioning membranes are probably involved in providing the energy required for salt loading of the channels. Other ultrastructural similarities of the basal cells of *S. virginicus, Spartina, Cynodon* and *Distichlis* include the presence of abundant ribosomes, occasional dictyosomes, elements of endoplasmic reticulum, small vacuoles and some rudimentary plastics.

The ultrastructure of the cap cell in *S. virginicus* is similar to those described for *Spartina, Cynodon* and *Distichlis* (Levering & Thomson 1971; Oross & Thomson 1982a,b; 1984) in lacking partitioning membranes. However, the cap cells of the microhairs of some grasses such as, *Enneapogon nigricans* and *Amphipogon caricinus* possess a system of membrane-bound channels, vesicles and associated microtubules (Amarsinghe & Watson 1988).

Small vacuoles, observed in the cap cells of *S. virginicus, Cynodon* and *Distichlis*, but not in *Spartina*, have been implicated in mechanisms of secretion (Thomson *et al.* 1969; Campbell & Thomson 1976). In the glands of several species, including *S. virginicus*, the cell organelles appear to be concentrated close to the outer surface of the cap cell, thereby suggesting that this region represents an area of intense metabolic activity (Oross & Thomson 1982a). The cuticular cavity observed in *S. virginicus* is similar to those in *Spartina, Cynodon* and *Distichlis* (Thomson 1975) and probably represents a temporary collecting compartment where secreted salts accumulate prior to elimination from the leaf (Campbell & Thomson 1976). The absence of a cuticle over the cap cells of most glands suggests that salt secretion may be associated with the rupture of the cuticle. Absence of a cuticle over the gland was not an artefact of sample preparation.

This study has shown that the bicellular salt glands of *S. virginicus* are especially adapted for salt secretion. The voluminous, embedded basal cell, with its elaborate membrane system and associated mitochondria, appears to access ions from surrounding mesophyll and epidermal cells and channels them symplastically to the cap cells. In the latter, the ions seem to be compartmentalised in small vacuoles and transported to the cuticular cavity, prior to exclusion from the leaf either through cuticular pores or rupture of the cuticle.

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