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Wall protuberance formation and function in secreting salt glands of *Tamarix aphylla* L.

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Salt glands of *Tamarix aphylla* consist of three pairs of secretory cells arranged one upon the other. At the stage of secretion, the upper and middle pair of secretory cells develop in their walls an internal system of anastomosed rods, the protuberances. In the formation of the wall protuberances, Golgi vesicles and microtubules appear to participate. The stage of salt secretion is also characterized by the presence of numerous mitochondria and microvacuoles. Microvacuoles contain the secreted solution and accumulate in the region of the wall protuberances. The interaction between microvacuoles and wall protuberances as well as the genesis of wall protuberances constitute new findings on the subject.

Keywords: *Tamarix aphylla*, salt glands, wall protuberances, microscopy, SEM, TEM

Introduction

Wall protuberances are rod-like projections of the cell wall towards the cytoplasm. They may be single or branched and may develop in the vicinity of the wall or extend deep into the cell interior. Cells bearing wall protuberances are considered to participate in short-distance transportation of solutes (GUNNING and PATE 1974). Wall protuberances have been observed in angiosperms (MARINOS 1970), ferns (GUNNING and PATE 1969), bryophytes (DUCKETT et al. 1977), algae (FRANCESCHI and LUCAS 1980), etc. More specifically, they have been localized in the stem (PATE et al. 1970), the root (KRAMER et al. 1977), the leaf (EVERT 1980), the flower (PETERSON et al. 1979), the fruit (COCHRANE and DUFFUS 1980), the seed (NEWCOMB 1978), the root nodule (NEWCOMB et al. 1977), the mycorrhiza (HADLEY et al. 1971), etc. As concerns the type of tissue, wall protuberances have been identified in the epidermal tissue (BIRCH 1974), the ground tissue (BUTTERFIELD et al. 1981), the secretory tissue (SCHNEPF and PROSS 1976, ROZEMA et al. 1977) and particularly, the conductive tissue (BOWES 1973, PETERSON and YOUNG 1975). The bulk of publications dealing with wall protuberance-bearing cells appeared in the seventies and many issues became resolved, and yet questions referring to their formation and development remain, to our knowledge, open.

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The present work provides ultrastructural evidence for the genesis of wall protuberances and their functional association with the secreted microvacuoles in the salt glands of *Tamarix aphylla*.

Materials and methods

For light microscopy (LM) and transmission electron microscopy (TEM), small segments of *Tamarix aphylla* L. leaves were initially fixed for 4 h with 3% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2. After washing in buffer, the segments were postfixed for 3 h with 2% osmium tetroxide (similarly buffered) and then dehydrated in an ethanol series. Dehydration was followed by infiltration and embedment in Spurr's resin. Semithin sections for LM were obtained in a Reichert Om U_2 ultramicrotome, stained with toluidine blue O and photographed in a Zeiss III photomicroscope. Ultrathin sections for TEM were cut in a Reichert-Jung Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate and examined in a JEM 2000 FXII transmission electron microscope.

For scanning electron microscopy (SEM), leaf segments, after fixation and dehydration, were critical point dried in a Balzers CPD 030 device and then coated with carbon in a JEE-4X vacuum evaporator. Observations were made in a JSM 840-A scanning electron microscope.

Results

The salt glands of *Tamarix aphylla* are epidermal structures located on both surfaces of the leaf (Figs. 1 A, B). Under the scanning electron microscope, they appear as local dome-like projections which are clearly distinguished from the surrounding typical epidermal cells (Fig. 1 A, asterisk). In leaf cross-sections, a developed salt gland seems to consist of three pairs of secretory cells arranged one upon the other (Fig. 1 B). The cells increase in size towards the top of the gland and their nuclei are large and centrally located.

Light microscopical examination of secreting salt glands reveals that the prominent characteristics associated with secretion are the raising of the cuticle from the apical walls (Fig. 1 B, arrow) and the presence in these walls of internal protrusions (Fig. 1 B, arrowheads). Electron microscopical observations show that in the outer pair of secretory cells, the walls (particularly the apical walls) locally extend towards the cytoplasm forming a system of rod-like structures, the protuberances (Fig. 1 C). Protuberances may penetrate the cytoplasm as single rods, or may branch and anastomose, forming an elaborate interconnected system. In the region of the protuberances, numerous mitochondria occur. High magnification of the protuberances reveals that they have a fine granular constitution and their structure is more compact than that of the normal wall (Fig. 1 D). The protuberance rods were measured to have an average thickness of 120 nm.

Protuberances do not exist until divisions of secretory cells in the salt glands are completed. When a gland becomes fully formed and salt secretion initiates, protuberances start to develop in the secretory cells, an indication that their presence is directly associated with the secretory process. In the middle pair of secretory cells, wall protuberances are not restricted to the periphery of the cells but often extend deep into the innermost cytoplasmic region (Fig. 2 A).

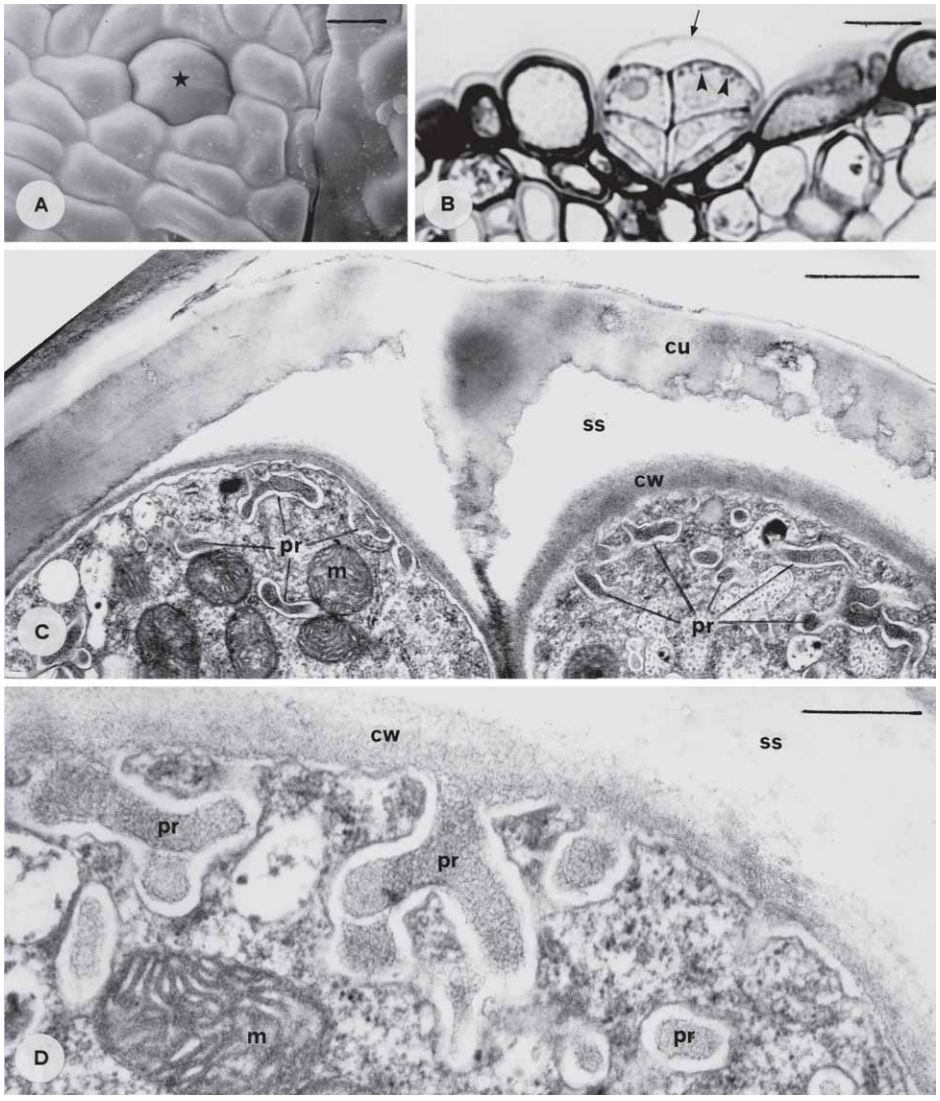

Fig. 1. Light, SEM and TEM micrographs illustrating salt glands. **A** – SEM micrograph illustrating a salt gland (asterisk) on the leaf surface; **B** – LM micrograph of a secreting salt gland. The cuticle (**arrow**) is detached from the apical cell walls, which bear a system of internal protuberances (**arrowheads**); **C** – TEM micrograph taken at the upper part of a salt gland. The cuticle (**cu**) is raised forming a subcuticular space (**ss**). The apical walls (**cw**) bear towards the cytoplasm many anastomosed protuberances (**pr**) **m**=mitochondrion; **D** – Higher magnification of the protuberances. They exhibit a fine granular substructure, denser than that of the typical wall. Bars: 12 μm (A), 10 μm (B), 1 μm (C), 0.4 μm (D).

The initial stages of protuberance formation have been identified and followed. Thus, at the wall point where a protuberance is formed, an accumulation of vesicles and short elements of the rough endoplasmic reticulum takes place (Fig. 2 B)., Microtubules appear to

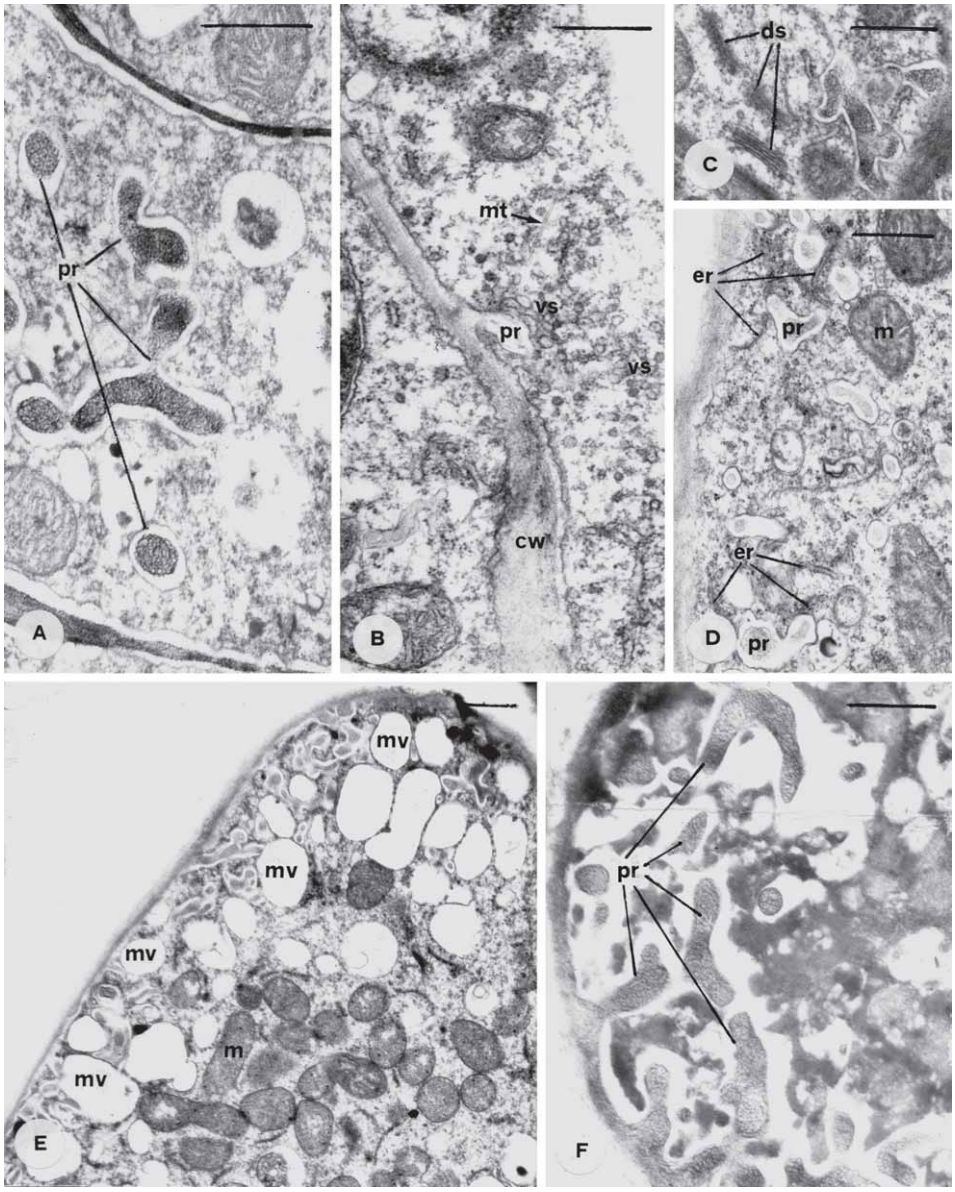

Fig. 2. TEM micrographs showing the development and function of wall protuberances in a secreting salt gland. **A** – Wall protuberances (**pr**) extending deep into the cytoplasm of the middle pair of secretory cells; **B** – Early stage of a protuberance formation. At the region of the protuberance (**pr**), many vesicles (**vs**) and converging microtubules (**mt**) appear; **C** – Active dictyosomes (**ds**) during the stage of protuberance formation; **D** – Endoplasmic reticulum elements (**er**) and mitochondria (**m**) in the vicinity or in contact with wall protuberances; **E** – Numerous microvacuoles (**mv**) accumulated along the apical wall protuberances in the upper pair of secretory cells. Note the presence of many mitochondria; **F** – A lysed secretory cell of a salt gland. The cytoplasmic components are disorganized, whereas the wall protuberances (pr) are well retained. Bar = 0.5 μm. Bars: 0.4 μm (A), 0.5 μm (B, C, D, F), 0.8 μm (E).

converge on this wall point. Vesicles most probably originate from the Golgi apparatus, since during this phase numerous active dictyosomes exist (Fig. 2 C). In the progress of protuberance development, mitochondria and endoplasmic reticulum elements approach the protuberances and occasionally come into contact with them (Fig. 2 D).

Apart from wall protuberances, the secretion stage is characterized by the presence of high numbers of microvacuoles and mitochondria (Fig. 2 E). Microvacuoles move in a great population towards the periphery of the secretory cells, where they become attached to the wall protuberances (Fig. 2 E). After secretion becomes completed, the secretory cells start to disorganize until they finally become fully lysed. At this stage, no organelles or other cytoplasmic components can be discerned and the whole cytoplasm has a dark foamy appearance. The only cellular element which retains its entity is the wall protuberance network (Fig. 2 F).

Discussion

When cell divisions in the salt glands of *Tamarix aphylla* are completed, the glands enter the secretory phase. The principal structural feature characterizing the stage of early secretion is the formation in the walls of the outer and middle pair of secretory cells of ingrowths penetrating the cytoplasm. These ingrowths (protuberances) are highly developed in the apical wall of the outer pair of cells, less so in the middle pair, whereas the inner pair of cells devoids of protuberances.

Wall protuberances constitute a typical trait of a type of cells called »transfer cells«, which are considered to be involved in the short distance flux of solutes (GUNNING and PATE 1974). The dual feature »wall protuberances-lining plasmalemma« is probably associated with both fast fluxing of solutes and fluxing of large amounts of solutes. These two parameters are quite important in:

1) Emergence of leaves. In spring, leaves emerge in a rather short time, and so large amounts of water and nutrients are immediately needed. These needs are covered with the formation and function of transfer cells in the conductive tissue.
2) Germination of seeds. The growth of the seed embryo into a seedling is relatively rapid, and a quick mobilization of nutrients from the endosperm/cotyledons takes place via transfer cells (GORI 1977).
3) Survival of halophytes. In the halophytes, the excess of the uptaking salty solution should be fast removed in order to prevent cell hyperosmosis. Salt removal is performed through salt glands, the secretory cells of which bear wall protuberances (ROZEMA et al. 1977, FARADAY and THOMSON 1986).
4) Pollination of flowers. When a flower opens, the process of pollination becomes immediately activated and floral nectaries start producing insect-attracting nectar. In order to facilitate secretion of large amounts of nectar within a short time (as long as flower opening lasts), nectaries bear numerous protuberances in their cell walls (FAHN 1979).

At the wall point where a protuberance starts to form, an accumulation of Golgi vesicles as well as converging microtubules were observed. This figure resembles that of the formation of the cell plate where Golgi vesicles are moved on phragmoplast microtubules via

motor proteins to the concrete region of cell wall formation (EVERT 2006). The complement of protuberance formation is followed by a great increase in the number of mitochondria and microvacuoles. Microvacuoles appear only in the upper pair of secretory cells and move towards the apical walls where protuberances are highly developed. There, they come into contact with the protuberance plasmalemma and by exocytosis release their content into the cell wall (and then into the subcuticular space). The content of the microvacuoles has been cytochemically identified as corresponding to the secreted salty solution (THOMSON et al. 1969).

The fact that the salt is secreted by microvacuoles and not in the form of free cytoplasmic molecules contradicts the generally received opinion that the increase of the plasmalemma surface at the protuberance region is related to an eccrine secretion (via molecules passing across the plasmalemma, DURKEE et al. 1981, NEPI et al. 1996). In that case, what is the advisability of such an increase of the plasmalemma surface? To this point the interpretation could be expressed that the high plasmalemma surface probably aims at creating a great number of disposable positions for the numerous microvacuoles, facilitating thus their fusion with the plasmalemma, a fact which would not be possible if a great number of microvacuoles became thickly crowded in a small plasmalemma surface. The network of wall protuberances is well retained even after the secretory cells become lysed during salt gland collapse. In other cell types under lysis, the cell walls appear disintegrated or refolded (BOSABALIDIS and TSEKOS 1986). This event stresses the important role the wall protuberances play in the process of salt secretion.

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