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Ultrastructural observations on foliar glandular trichomes of *Stevia rebaudiana*

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Foliar glandular trichomes of *Stevia rebaudiana* (Bert.) Bert. were examined by transmission electron microscopy in order to detect changes occurring in their cells in association with the secretory process. In the foliar, 10-celled lipophilic glandular trichome of *Stevia rebaudiana* (Bert.) Bert., the six secretory cells forming three pairs of head cells are vacuolated, possess large nuclei and are rich in ribosomes, mitochondria, plastids and ER elements. Dictyosomes are relatively frequent in these cells. The plastids, which form starch grains, are leucoplasts in cells of the apical pair and chloroplasts in cells of the two subapical pairs. The basal cells and stalk cells possess some degree of vacuolation and are rich in ribosomes. Also in these cells, the nuclei are relatively large; ER elements, chloroplasts and dictyosomes are present in moderate number, and mitochondria are frequent. Wall ingrowths are found in head cells as well as in the stalk and basal cells. Plasmodesmata, in moderate number, occur more frequently in transverse walls of head cells, as well as in those between cells of the second subapical pair and stalk cells and between the latter and basal cells. Plasmodesmata connect mesophyll cells and basal cells. To form the secretory sheath, the cuticular membrane detaches from the outer walls of the apical secretory cells, along a line that appears to be the pectin layer.

Introduction

The foliar, 10-celled biseriate glandular trichome of *Stevia rebaudiana*, the object of this study, has previously been described by Monteiro *et al.* (2001) with regard to its development and basic histochemical aspects.

Material produced by secretory cells of the *S. rebaudiana* glands, and stored in the trichome cavity is lipophilic; it also possesses a light hydrophilic character (Monteiro *et al*. 2001). The same material is mostly lipophilic, according to Cornara *et al*. (2001). Results from the HRGC/MS analysis carried out by these authors on essential oil obtained from leaves and inflorescences of *S. rebaudiana* plants reveal sesquiterpenes as the most abundant class of compounds, their levels being 6- to 7-times greater in the inflorescences than in the leaves.

The main objective of the present investigation on the *S. rebaudiana* glandular trichome was to detect the ultrastructural modifications shown by its cells at the phase of secretion, and to compare such modifications to those occurring in other lipophilic glands, especially trichomes. Among the studies to be taken into consideration for such comparison are those by Vermeer and Peterson (1979), Werker and Fahn (1981), Ascensão and Pais (1985), Oliveira and Pais (1990), Kim and Mahlberg (1991), Duke and Paul (1993), Figueiredo and Pais (1994), Afolayan and Meyer (1995), Ascensão *et al*. (1997), and Kim and Mahlberg (2000).

Materials and Methods

Vegetative shoot apices of *Stevia rebaudiana* (Bert.) Bert. (Asteraceae) with the upper third removed, and measuring 1.5–2.0mm in length, were fixed in 6.4% glutaraldehyde-10.0% acrolein solution (based on Vermeer and Peterson 1979) in 0.05M Na cacodylate buffer at pH 7.0, for 22h at 4°C. After fixation, they were washed for 30 minutes in three changes of buffer (0.05M Na cacodylate buffer, pH 7.0), and postfixed for 1h at room temperature in 2.0% osmium tetroxide solution in the aforementioned buffer. The specimens were then washed for 30 minutes in three changes of the same buffer, and for 30 minutes in three changes of deionised water. After these washings, they were dehydrated

in a graded ethanol-acetone series, and embedded in Spurr's resin. Thin sections were stained with uranyl acetate and poststained with lead citrate. Well-preserved glandular trichomes were examined in 150 out of 200 sections, in a Zeiss EM 900 transmission electron microscope at 50kV. The best trichomes for ultrastructural details were then photographed.

Results

General observations

The *Stevia rebaudiana* glandular trichome is formed by one pair of basal cells, another of stalk cells, and three pairs of head cells (Monteiro *et al*. 2001). In the present paper, the head cell pairs are referred to as apical, first subapical, and second subapical cell pairs, the latter being basipetally followed by the stalk cells.

Head cells

Trichome cavity still to be formed — The cuticular membrane (Holloway 1982) on the trichome head shows a gradual increase in thickness from the cells of the second subapical pair to those of the apical pair (Figure 1a–c).

Head cells are vacuolated (Figure 1d–i), possess large nuclei (Figure 1d), and a great number of free ribosomes (Figure 1a–k). Dictyosomes and plastids are more frequent in the cells of the apical pair. However, in general the presence of these organelles and also of mitochondria, as well as of rough and smooth endoplasmic reticulum (RER and SER, respectively), can be described as modest in head cells (Figure 1d–k; SER not visible in the micrographs). In the plastids, the number of thylakoids decreases from the cells of the second subapical pair to those of the apical pair (Figure 1d–i). In the latter, the plastids, as is typical of leucoplasts, are practically devoid of an internal membrane system (Figure 1b, 1f–i). Such a system is distinct, although poorly developed, in plastids of the cells of the first and second subapical pairs; these organelles can be considered to be chloroplasts (Figure 1d). Occurrence of starch grains is sporadic in chloroplasts. Such grains are, however, relatively evident in leucoplasts of the cells of the apical pair; besides starch, some of these plastids possess a very electron-dense stroma (Figure 1f–i).

Plasmodesmata (one of them is seen obliquely sectioned in Figure 1e), and discrete ingrowths (Figure 1a–e, j and k) are present in the cell walls.

Trichome cavity present — General views, as well as details of head cells of glandular trichomes in which the cavity has already been formed, are seen in Figures 2–5.

The cavity shown by the trichome of Figure 2a–c is seen to be still incomplete on one side (a, b), and complete on the opposite side (c), in which the level of the basal limit of the cavity does not surpass that of the lower region of the cells of the apical pair. A detail of Figure 2a is shown in Figure 2b. In the latter, the cuticular membrane is detaching from the cell wall, resulting in the formation of the trichome cavity. This detachment occurs along a region that corresponds to that believed to contain pectin (pectin layer, Holloway 1982).

The cuticular membrane on head cells of the glandular trichome is conspicuously thicker than that on cells of a nonglandular trichome (Figure 2b–c).

The glandular trichome of Figures 2d–f and 3a–h, and the other seven glandular trichomes, shown in Figures 3 (3i; 3j), 4 (4a–e; 4f–h; 4i), and 5 (5a and 5b; 5c–g) possess certain features indicating involvement with secretory activity. During a certain period of the trichome life, the cuticular membrane remains attached to the cell wall at the tip of the trichome in the region between the two cells of the apical pair. This is illustrated by Figures 2f and 5b.

Head cells of a glandular trichome are seen in general view in Figure 2d–f, and in details in Figure 3a–h. Details of two other glandular trichomes are shown in Figure 3i and 3j. Figure 3a–h includes parts of cells of the second and first subapical pairs in a–d; parts of the latter and of cells of the apical pair in e and f; and parts of cells of the apical pair, only, in g and h. The number of plastids, mitochondria, elements of the RER and SER, and dictyosomes in all head cells is found to be higher, if compared with that of the earlier phase (Figure 1a–k) when the trichome cavity is still to be formed. The cells of the apical pair are the most significant among the head cells regarding the number of each type of the aforementioned organelles (Figure 2d–f). Some plastids (Figure 2d and 2f) are amoeboidal in shape especially in the cells of the apical pair. Starch grains are very frequent in these cells, and modest in quantity in the cells of the first and second subapical pairs (Figure 2d–f). Those features concerning the internal membrane system of the plastids, and characterisation of leucoplasts (in cells of the apical pair) and chloroplasts (in cells of the first and second subapical pairs), as pointed out for the preceding phase, are now more evident. This may be seen in Figure 2b and in the general views of the head cells shown by Figure 2d–f. Some ER elements (mainly RER) are in a periplastidal position (Figure 3c, 3g and 3i), and some RER elements seem to be associated with mitochondria (Figure 3e and 3f). Vesicular and nonvesicular structures (Figures 2b, and 3b–e, 3h and 3i) can be found situated close to or even touching the plasmalemma. Many of them are believed to be portions of ER elements which were variously (including transversely) sectioned on the ultramicrotome. Also, vesicles of dictyosomal origin may be among these structures, since it is possible to see dictyosomes (as well as vesicles associated with them) situated close to cell walls (Figures 2b and 3h).

Abbreviations and symbols (all Figures): AP, cell of the apical pair; BC, basal cell; CM, cuticular membrane; CW, cell wall; D, dictyosome; LW, lateral wall; MC, mesophyll cell; N, nucleus; NT, nonglandular trichome; Pm, cell wall with plasmodesmata; Pt, plastid; RER, rough endoplasmic reticulum; SC, stalk cell; SER, smooth endoplasmic reticulum; SP1, cell of the 1st subapical pair; SP2, cell of the 2nd subapical pair; TC, trichome cavity; star (Figure 2d and 2f), amoeboidal plastid; arrow (Figure 3c, 3g and 3i), endoplasmic reticulum tending to be in periplastidal position; broad short arrow (Figure 3e and 3f), endoplasmic reticulum positioned very close to mitochondrion; triangle (Figure 3i) and arrowhead (Figure 4g and 4i), fine layer of materials released from the outer walls of apical cells; thin short arrow (Figure 4a–c), wall ingrowth — a reference point characterizing the wall between SP1 and SP2; asterisk (Figure 4g–i), presence of signs of degeneration in the cell; white short arrow (Figure 5a), one of the little deposits believed to be formed by phenolic compounds

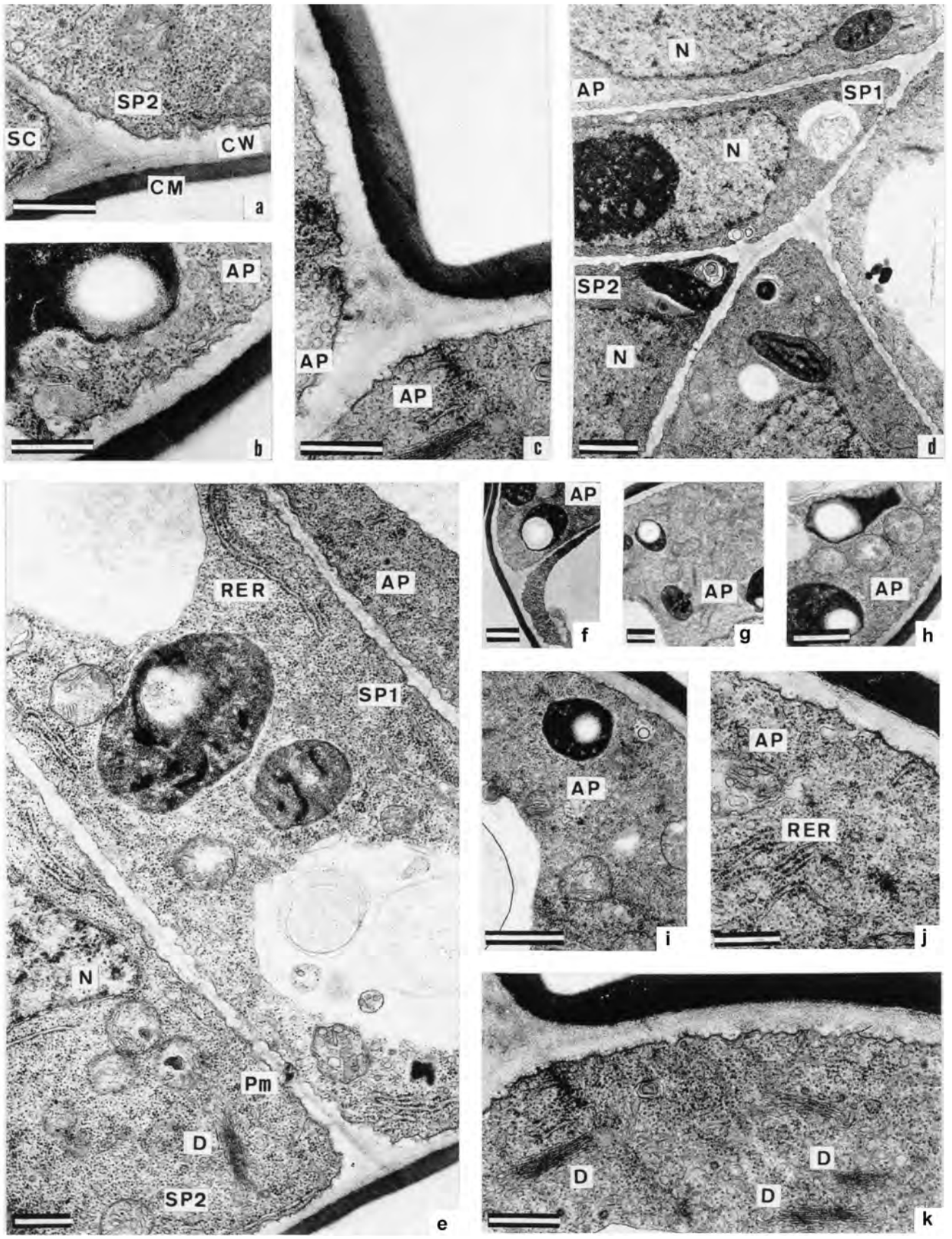

Figure 1a–k: Aspects of a glandular trichome in which the cavity is still to be formed. Head cells. Detailed views in: a, b, c, e, i, j and k. Bar = 0.5µm (a–c, e, j, k) and 1.0µm (d, f–i)

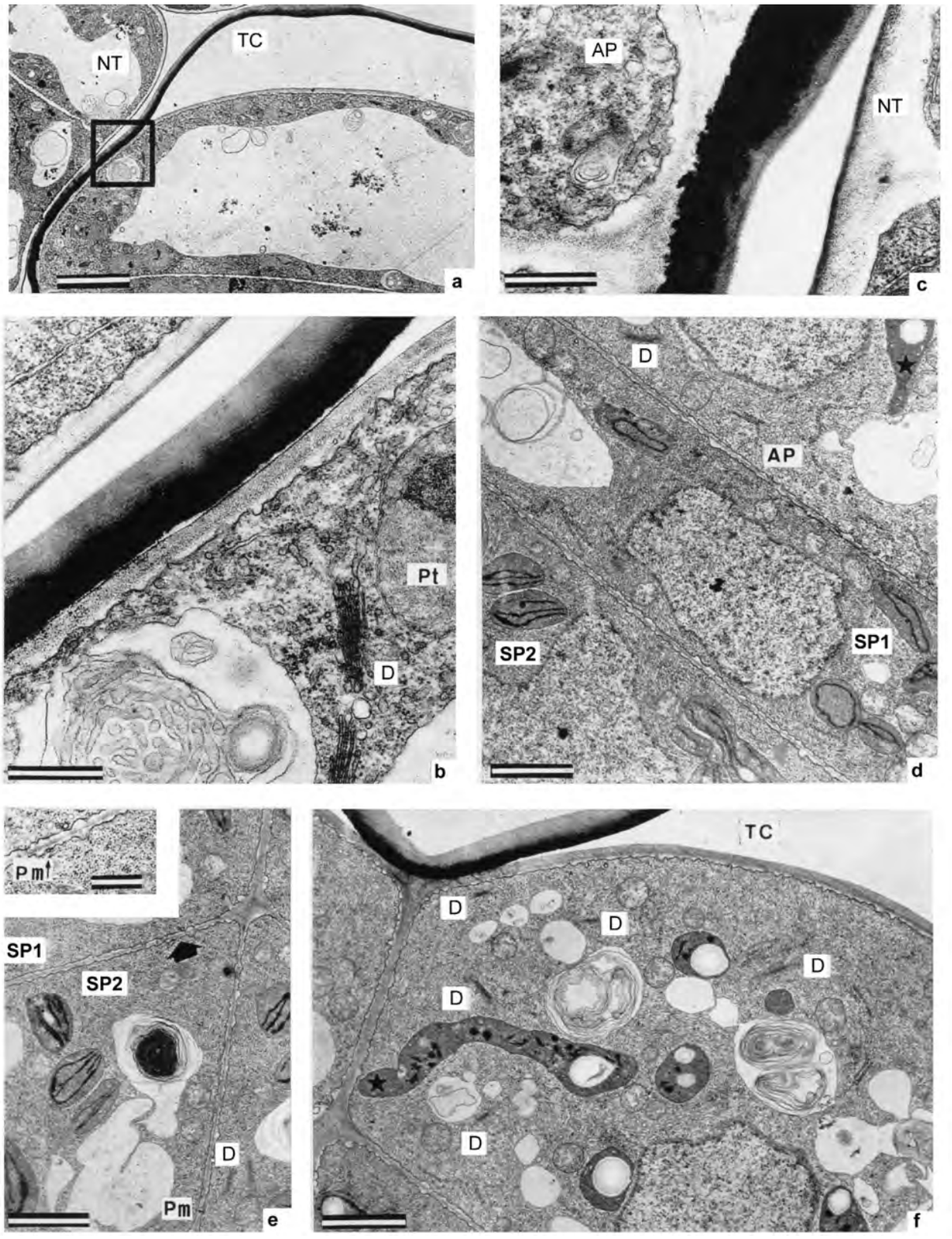

Figure 2a–f: Aspects of two glandular trichomes with cavity present. (a–c) Glandular trichome in lateral view. (a) A cell of the apical pair. The square delimits the region in which the cuticular membrane detaches from the cell wall. Included is part of a nonglandular trichome. (b) Detailed view of the aforementioned region. (c) Apical cell of a and b, opposite side. (d–f) Head cells of another glandular trichome — face view. In e the region indicated by the broad short arrow in the cell wall is shown in the inset. Bar = 4.0μm (a), 2.0μm (d–f) and 0.5μm (b, c, inset in e)

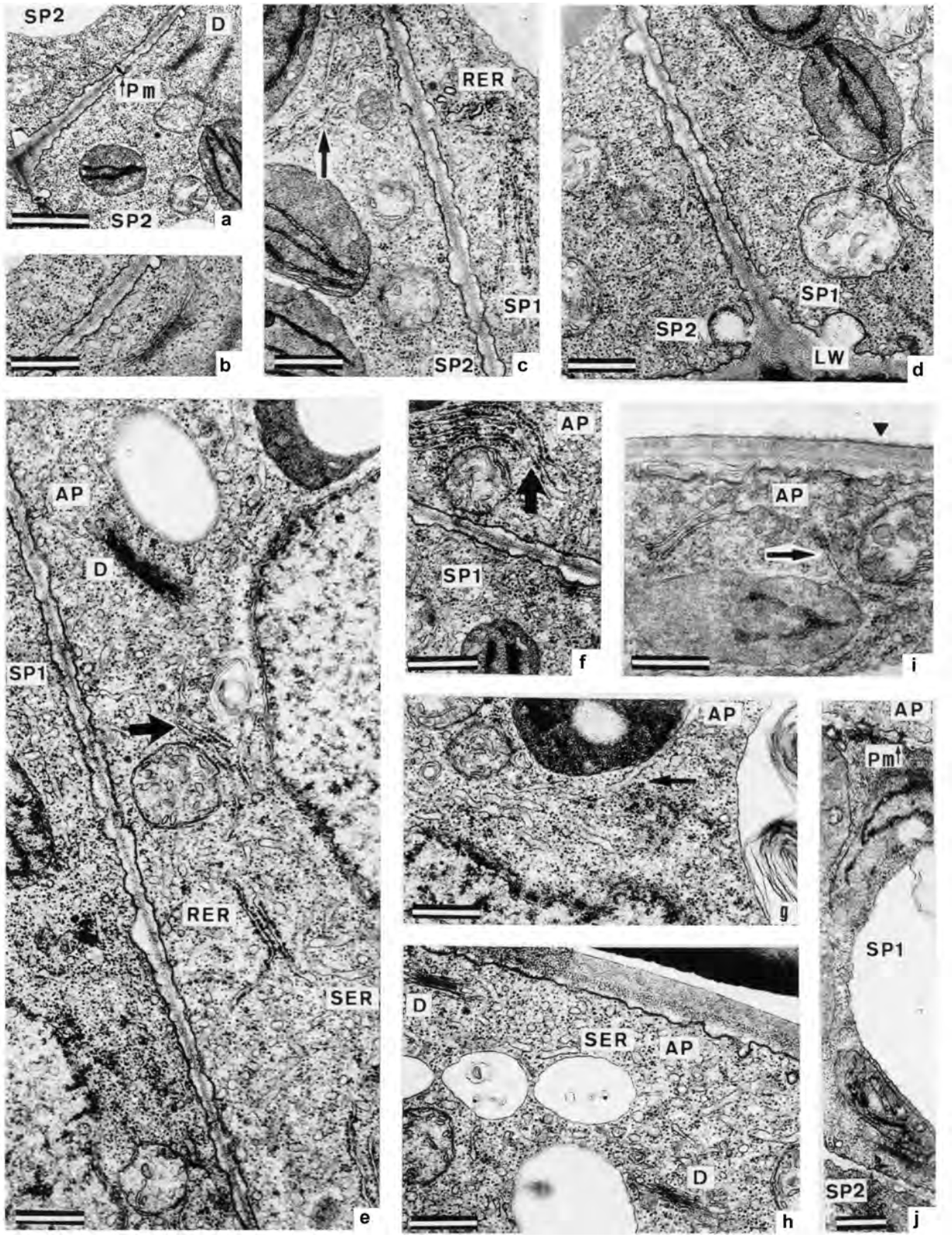

Figure 3a–j: Aspects of three glandular trichomes with cavity present. (a–h) Glandular trichome shown in Figure 2d–f. (a and b) Cells of the 2nd subapical pair — detailed view in b. (c–h) Other detailed views of head cells. (i) Cell of the apical pair of another glandular trichome. (j) Head cells of another glandular trichome. Bar = 1.0µm (a) and 0.5µm (b–j)

Existence of plasmodesmata is noticed between cells of the second subapical pair and those of the first subapical pair (Figure 2e), between the latter and cells of the apical pair (Figure 3j), and also between cells of the same pair (in anticlinal walls), e.g. the second subapical pair of Figure 2e (bottom), seen also in detail in Figure 3a. Plasmodesmata are not numerous. In the periclinal cell walls they are more frequent than in the anticlinal ones.

Ingrowths originated by lateral and transverse cell walls, are more numerous and more developed than those seen in the preceding phase; many of them indicate a recent addition of wall building materials (Figures 2d–f, and 3b–f and 3h).

Figure 3i shows fine wall materials being released from the surface of the apical cell.

Myelin figures are common in vacuoles (Figures 2d–f, and 3g and 3h).

Secretory activity associated with the three kinds of head cells are seen in the four glandular trichomes of Figures 4 (a–e, f–h, i) and 5 (a and b). In these trichomes, the cuticular membrane is still attached to the region between the two apical cells as shown by Figure 5b, and granular osmiophilic material is found in their outer walls (Figures 4d, 4g, 4i, and 5a and 5b). Also, some fine wall materials released from the periphery of the cells of the apical pair, form a discrete layer on their surfaces (Figure 4g and 4i).

The micrographs that compose Figure 4 show cell details in the second subapical pair (b and c in the a–e trichome; and f in the f–h trichome), first subapical pair (c in the a–e trichome; and f in the f–h trichome), and apical pair (e in the a–e trichome; g and h in the f–h trichome, and i in the i trichome). The ER (mainly the RER) is highly proliferated (Figure 4b, 4c and 4f–i). Figure 4 shows examples of periplastidal ER in b and c. Well-developed dictyosomes are still common in the cells (Figure 4b, 4f and 4i). The previously mentioned vesicular and nonvesicular structures, frequently situated close to or even touching the plasmalemma, are seen in great numbers (Figure 4b, 4c, 4f, 4g and 4i). The plastids, with a poorly-developed internal membrane system (Figure 4b, 4c, 4e, 4f, 4h and 4i), are also numerous, and possess large starch grains (Figures 4a–e and 5a).

Wall ingrowths are still conspicuous (Figure 4b, 4c, 4f and 4i).

Certain features noticed in these trichomes, indicate that they are in a late developmental phase, and already starting to senesce . The cells of the apical pair, are the first to show such features. Degenerative changes occur in the organelles of these cells: a great number of ER elements are distorted and ill-defined (Figure 4g–i). Alterations in structure are also present in some plastids (Figure 4h and i), and in some mitochondria (Figure 4g–i). In Figure 5a, there are small deposits of electron-dense substances in the cells of the apical pair. These substances, believed to be phenolic compounds, increase in amount in the whole trichome as it ages (Figure 5b).

The cuticular membrane of the trichomes in which the senescent phase is already established is no longer attached to the region between the two cells of the apical pair (Figure 5c–g). The basal limit of the trichome cavity is at the level of the lower portion of the cells of the apical pair (Figure 5c). The same senescent trichome also contains materials inside its cavity. Figure 5d–g shows the formation of these materials: compact bodies, loosely aggregated masses and also membrane-like formations. The latter are visible in the periphery of the compact bodies, as well as in the periphery of apparently empty spaces. Part of the materials are also seen dispersed in the trichome cavity. Figure 5d–g suggests that the cavity contents are degrading, and that they originate from a sloughing-off (still discrete in appearance in Figures 3i, and 4g and 4i) of the outer walls of the cells of the apical pair. Consequently, such materials occur in the trichome cavity together with the secretion products (not visible in the micrographs).

Basal cells and stalk cells

Trichome cavity present — Figure 6a–f illustrates basal and stalk cells of two glandular trichomes. One of these trichomes is seen in a in general view, and the other in b–f. In the latter sequence, a general view is shown in b, and details in c–f. Observations concerning head cells of these trichomes are illustrated by Figures 2d–f and 3a–h for the trichome of Figure 6a, and by Figure 4f–h for that of Figure 6b–f.

The basal cells and the stalk cells show some degree of vacuolation (Figure 6a and 6b) and are rich in free ribosomes (Figure 6a and 6c–f); and their nuclei are large (Figure 6b, 6d and 6e). Also, the dictyosomes, ER elements and plastids occur in moderate number, and mitochondria seem to be numerous (Figure 6a and 6c–e). Vesicles associated with the dictyosome of Figure 6e appear to be adding building material to a transverse wall of one of the stalk cells. Regarding the degree of development of the internal membrane system, the plastids of the basal cells are similar to those of the leaf chlorenchyma (Figure 6c and 6d), while the plastids of the stalk cells can be considered to be intermediate between the plastids of the basal cells and those of the head cells of the second subapical pair (see plastids, comparing Figure 6a and 6e to Figures 2d and 3c).

Relatively frequent plasmodesmata occur between the basal cells and stalk cells, and between the latter and the head cells of the second subapical pair (Figure 6a and 6e). They are also found between mesophyll cells and the basal cells (Figure 6c). Ingrowths are present in the transverse and lateral walls of the basal and stalk cells (Figure 6a and 6e).

Figure 6f shows that the cuticular membrane on both the basal and stalk cells is thicker and much less cutinised than that on a cell of a nonglandular trichome. The same illustration also shows that the degree of cutinisation in the basal cell is relatively conspicuous in the region where the cuticular membrane is continuous with that of the basis of the nonglandular trichome. Such cutinisation decreases in degree upwards.

Discussion

Confirming previous observations (Monteiro *et al*. 2001), the basal limit of the secretory sheath in the *Stevia rebaudiana* glandular trichome extends to the level of the lower portion of the apical cell pair. Therefore, the apical cells may be the only ones able to secrete material into the trichome cavity. In

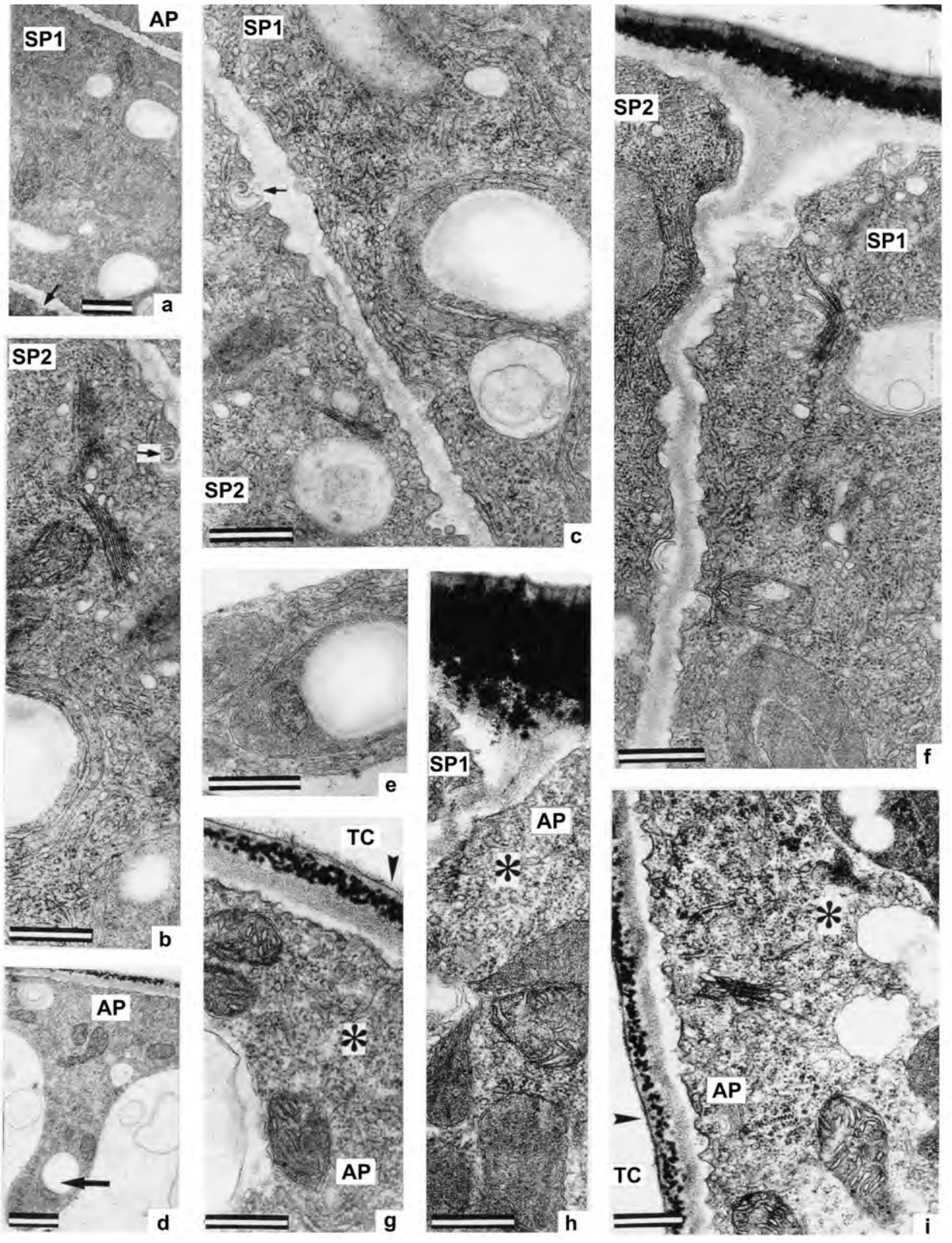

Figure 4a–i: Aspects of three glandular trichomes with cavity present. Granular osmiophilic material occurs (d, g and i) in the outer walls of cells of the apical pair. (a–e) Head cells of a glandular trichome — detailed views in b, c and e. Straight arrow in d indicates the region seen in e. (f–h) Head cells of another glandular trichome. (i) Cell of the apical pair of another glandular trichome. Bar = 1.0μm (a, d) and 0.5μm (b, c, e–i)

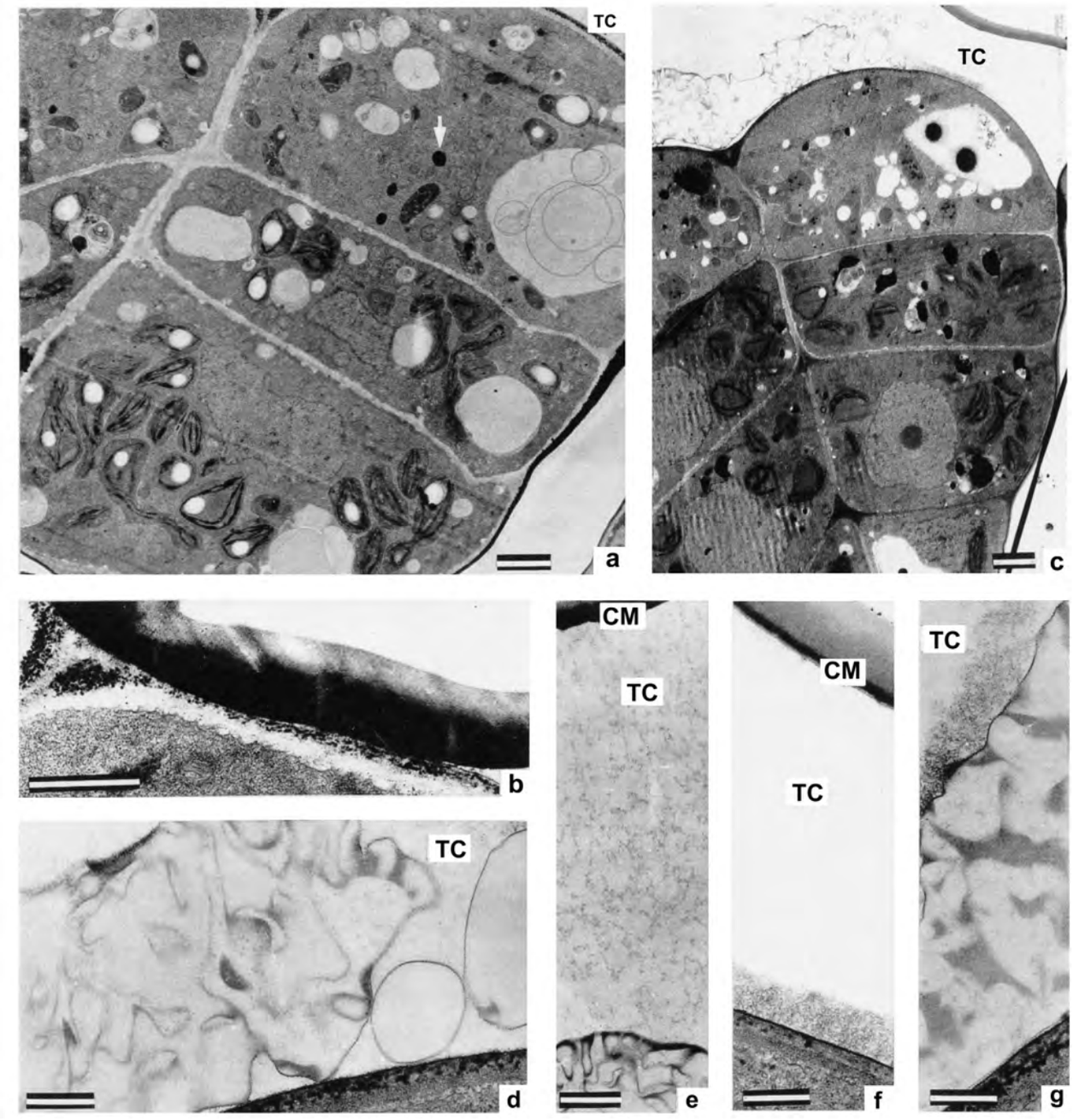

Figure 5a–g: Aspects of two glandular trichomes with cavity present. Granular osmiophilic material occurs (a, b, d, f and g) in the outer walls of cells of the apical pair. (a) Head cells of a glandular trichome. (b) Apical region of the same trichome. (c) Head cells of another glandular trichome, senescent phase. (d–g) Cavity contents of the trichome in c. Bar = 2.0µm (a, c), 1.0µm (b, e) and 0.5µm (d, f, g)

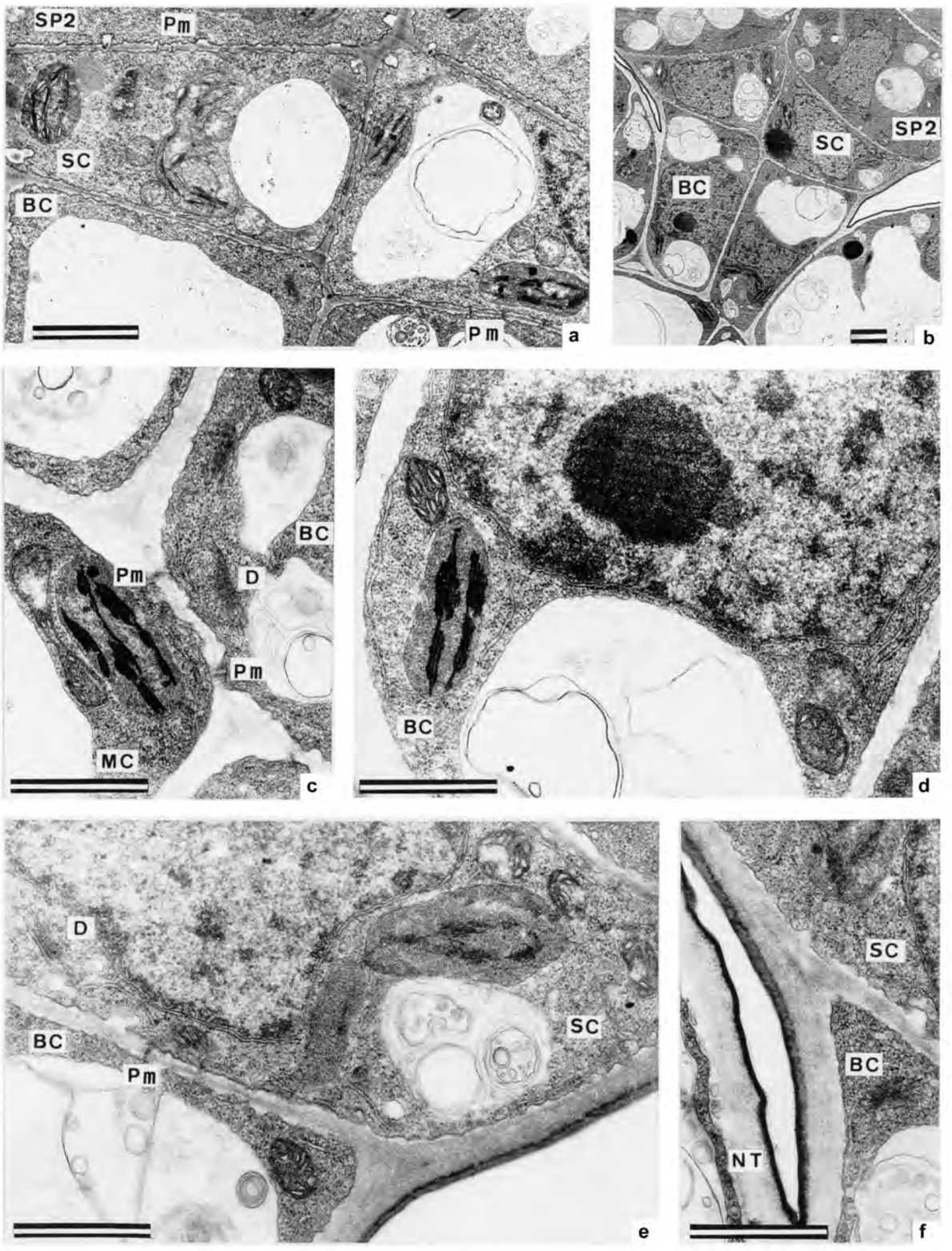

Figure 6a–f: Aspects of two glandular trichomes. (a) Basal cells and stalk cells of the glandular trichome shown in Figures 2d–f and 3a–h. (b–f) Part of the glandular trichome shown in Figure 4f–h. (c) Mesophyll cell and basal cell (the latter on the right side of the trichome shown in b). (d) Basal cell (left side of the trichome in b). (e) Basal cell and stalk cell (right side of the trichome in b). (f) Basal cell and stalk cell beside a nonglandular trichome (the latter on the left side in b). Bar = 2.0µm (a, b) and 1.0µm (c–f)

Chrysanthemum morifolium (Vermeer and Peterson 1979) and *Artemisia annua* (Duke and Paul 1993), the level attained by the basal limit of the secretory sheath is found at the upper portion of the first subapical cell pair and lower portion of the second subapical cell pair, respectively. In *S. rebaudiana*, the top region between the apical cells is the point at which the secretory sheath finishes its detachment from those cells. An opposite situation appears in *Artemisia annua* (Duke and Paul 1993) and *Chrysanthemum morifolium* (Figure 36 in Vermeer and Peterson 1979), where the abovementioned region is that in which the secretory sheath becomes independent.

Previous histochemical results (Monteiro *et al*. 2001) and the present study indicate that in the *S. rebaudiana* trichome, the secretory sheath basically is the cuticular membrane, detached from outer walls of the apical cells along a line described by Holloway (1982) as a pectin layer. In *Chrysanthemum morifolium* the structural composition of the secretory sheath and the way by which the trichome cavity is formed are similar to that is found in *S. rebaudiana* (Figures 32 and 34 in Vermeer and Peterson 1979). In *Cannabis sativa* (Kim and Mahlberg 1991, 1997), *Humulus lupulus* (Kim and Mahlberg 2000) and *Leonotis leonurus* (Ascensão *et al*. 1997), the internal part of the secretory sheath contains material from the outer walls of secretory cells. A possible rupture of the secretory sheath, and consequent release of the secretion product from the trichome cavity has been reported by Cornara *et al.* (2001).

The cuticle covering the *S. rebaudiana* trichome is relatively thick, as seen in *Leonotis leonurus* by Ascensão *et al*. (1997).

Variously developed wall ingrowths are present in all head cells of the *S. rebaudiana* trichome, occurring from one cell to another as well as from apical cells into the trichome cavity. These structures are also developed by the basal and stalk cells. Such protrusions, found in apical cells of lipophilic trichomes of other species, are thought to be associated with the transfer of secretory material into the trichome cavity (Vermeer and Peterson 1979, Werker and Fahn 1981, Duke and Paul 1993, Afolayan and Meyer 1995).

The *S. rebaudiana* glandular trichome exhibits the frequently emphasised characteristics of lipophilic glands: a well-developed ER (with predominance of RER at the secretory phase) and a great number of plastids (Fahn 2000). The relevance of the ER and, especially, of the plastids to the synthesis of terpenoids, has been shown in various biochemical studies, in some cases associated with TEM observations (Gleizes *et al*. 1980, Curry 1987, Nielsen *et al*. 1991, Gershenzon and Croteau 1993, McCaskill and Croteau 1995, Turner *et al*. 1999, 2000).

Generally, in many glands secreting lipophilic substances, the ER is thought to take part not only in the synthesis of such substances, but also in the intracellular transport of them (Fahn 2000). For this transport, the direct or indirect connection of ER elements to plastids (and other organelles) is considered to be important. In this intracellular transport, the final destination should be the plasmalemma with which the ER elements fuse, carrying out the granulocrine mechanism of elimination of secreted material from the protoplast. In glandular cells of the *S. rebaudiana* trichome, ER elements frequently are very close to the leucoplasts in the cells of the apical pair, and also surrounding chloroplasts of cells of the two subapical pairs. ER elements are also found near the plasmalemma, and appear to touch it in certain points. These features have also been described in studies on glandular trichomes of other species of the Asteraceae (Vermeer and Peterson 1979, Werker and Fahn 1981, Ascensão and Pais 1985, Duke and Paul 1993).

Occurrence of leucoplasts in cells of the apical pair, and chloroplasts in those of the subapical pairs, as seen in *S. rebaudiana*, has also been reported in the Asteraceae for *Chrysanthemum morifolium* (Vermeer and Peterson 1979), *Inula viscosa* (Werker and Fahn 1981), *Artemisia campestris* (Ascensão and Pais 1985) and *Artemisia annua* (Duke and Paul 1993). In the *S. rebaudiana* trichome, the chloroplasts of the cells of the second subapical pair are more differentiated in their internal membrane system than those of the cells of the first subapical pair. Differences in both the degree and type of differentiation in plastids have been seen in the *Inula viscosa* trichome by Werker and Fahn (1981). As in *S. rebaudiana*, the degree of such differentiation increases towards the trichome basis.

Starch grains are found in great quantity in all head cells of the glandular trichome of *S. rebaudiana*. For the *Artemisia annua* trichome, in which formation of starch grains has not been detected, Duke and Paul (1993) believe that during the secretory phase, the gland chloroplasts are able to convert photosynthate into terpenoids, and to export these to the cytoplasm. We can speculate, in the case of the *S. rebaudiana* trichome, that terpenoids (or their precursors) may be synthesised in leucoplasts of the cells of the apical pair, as well as in chloroplasts of the cells of the two subapical pairs. The substrate represented by photosynthates, later to be converted into terpenoids, should normally be formed in chloroplasts of the cells of the two subapical pairs, and part of these compounds may be translocated to the leucoplasts of the cells of the apical pair which, structurally, do not carry out photosynthesis. Excess photosynthate should account for the formation of starch grains in the plastids. The substrate may also be translocated from cells situated below those of the trichome head, including the leaf mesophyll cells. The presence of plasmodesmata and cell wall ingrowths should provide greater efficiency to the intercellular transport process of the materials. In addition, certain features (such as wall ingrowths, nuclei of large size, relatively numerous mitochondria, etc.), found in basal and stalk cells of the *S. rebaudiana* trichome, indicate that these cells are involved in some kind of metabolic activity related to the secretory process. Characteristics of intense activity found in basal and stalk cells of the peltate trichome of *Mentha* x *piperita,* led Turner *et al*. (2000) to suggest that such cells might play a role in the supply of carbon substrates to the nonphotosynthetic disc cells during secretion. Probably, starch grains amass in the gland as it gradually loses its ability to carry out the secretory process, as suggested by Akers *et al*. (1978).

Dictyosomes are relatively numerous in the *S. rebaudiana* glandular trichome, and are commonly found, along with dictyosome-derived vesicles, close to cell walls. They should be involved in secretion of polysaccharides, in this way adding materials to the cell walls, as suggested by Werker and Fahn

(1981) and Figueiredo and Pais (1994). Detection of electron-dense material in dictyosomes and vesicles derived from them in some lipophilic types of glands (Oliveira and Pais 1990, Monteiro *et al*. 1999, Fahn 2000) indicates that they may play a role, in some way, in the secretory process of these glands. Still regarding the *Inula* trichome, the dictyosomes may also be involved in the synthesis of polysaccharides found in the secretory material (Werker and Fahn 1981), whilst in *Achillea millefolium* (Figueiredo and Pais 1994, Figueiredo *et al*. 1995) they may be involved in processes of glucosylation of terpenoid compounds occurring in the essential oil produced by glandular cells from cell suspension cultures.

The senescence phase of the *S. rebaudiana* trichome appears similar to that found in *Chrysanthemum morifolium* by Vermeer and Peterson (1979). Electron-dense substances appear in the trichome secretory cells; they may be phenolic compounds (Monteiro *et al*. 2001).

In *S. rebaudiana* the substances collected in the trichome cavity are lipophilic and also, moderately, hydrophilic (Monteiro *et al*. 2001). Maybe the latter character is due to an amassment and possible degradation of those wall materials, which continuously slough off from surfaces of the trichome apical head cells after detachment of the cuticular membrane.

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