ULTRASTRUCTURE OF THE EPITHELIAL CELLS AND OLEO-GUMRESIN SECRETION IN BOSWELLIA SERRATA (BURSERACEAE)

by

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SUMMARY

The ultrastructure of epithelial cells of oleo-gumresin ducts in *Boswellia serrata*, the source of Indian olibanum, is described. Oleo-gumresin ducts are present in primary and secondary phloem. The duct lumen forms an enlarged apoplastic space surrounded by epithelial cells. The epithelial cells are rich in dictyosomes, lipid bodies, mitochondria with dilated cristae, multivesicular bodies, osmiophilic materials, plastids and vesicles. Plastids have poorly developed internal membranes. Dictyosomes and plastids are possible sites of resin synthesis. The gum component of the exudate is formed in dictyosomes and from the outer layers of the inner tangential wall (wall facing the duct lumen). This wall is replenished from inside by the activity of dictyosomes. The secretory materials are transported to the apoplast by granulocrine and eccrine secretion. They migrate through the loose microfibrils of the inner tangential wall into the duct lumen. Rarely, epithelial cells of young ducts have rudimentary plasmodesmata on the inner tangential wall which may be channels for passage of secretory materials into the duct lumen.

*Key words:* *Boswellia serrata*, granulocrine and eccrine secretion, medicinal plant, Indian olibanum, oleo-gumresin, salai guggal, ultrastructure.

INTRODUCTION

*Boswellia serrata* Roxb. is a medium to large deciduous tree of the family Burseraceae, common on dry hills throughout India. This tree has been extensively felled and used for making pulp and paper in India. However, the stem of this tree exudes upon injury an important oleo-gumresin called Indian olibanum, Indian frankincense or salai guggal. This golden-yellow exudate is fragrant and solidifies to brownish yellow tears or crusts on the tree trunk. *Boswellia* is the only non-coniferous source of turpentine and rosin in India (Anonymous 1988). Salai guggal is chiefly used for making incense sticks. It is also used for preparing varnishes, high-grade paints, lacquers and printing ink. The defatted component of the exudate is used in the textile industry and in medicine as it possesses anti-inflammatory and anti-arthritic activity (Anonymous 1988). The bark and the gumresin are used for asthma, dysentery, ulcers, haemor-

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rhoids, skin diseases, fever, bronchitis, diaphoresis, convulsions, urethrorrhoea, orchiopathy, cough, stomatitis, syphilitic diseases, chronic laryngitis, jaundice and arthritis. A commercial product prepared from salai guggal called ‘care’ is marketed in India for use in relieving muscular pain.

There have been several studies on the structure of secretory ducts in plants (Wooding & Northcote 1965a, b; Fahn & Evert 1974; Fahn & Benayoun 1976; Setia et al. 1977; Benayoun & Fahn 1979; Joel & Fahn 1980a, b, c; G.M. Nair et al. 1983; M.N.B. Nair et al. 1983; Bhatt & Shah 1985; Bhatt 1987; Venkaiah 1990, 1992; Bhatt & Mohan Ram 1992; Nair 1995). However, ultrastructure and mode of secretion of oleo-gumresin in *Boswellia* have not been studied. The present investigation was done to better understand the possible site of oleo-gumresin formation and mode of its elimination from the protoplast in *Boswellia serrata*.

MATERIALS AND METHODS

The bark samples were collected and fixed in 4% neutral formalin for light microscopic studies. Sections were cut on a sliding microtome and stained with toluidine blue O for histological studies (O’Brien & McCully 1981). The lipids were localized using Sudan black B and insoluble polysaccharides by PAS reaction (Jensen 1962). For electron microscopy the material was fixed in paraformaldehyde glutaraldehyde in 0.02 M cacodylate buffer at pH 7.1 (Karnovsky 1965). The samples were post-fixed in 2% OsO₄ in cacodylate buffer for two hours. The specimens were then immersed in 2% uranyl acetate for 30 minutes, dehydrated in a cold graded acetone series, infiltrated and embedded in spurr (Spurr 1969). Sections were cut on a Reichert OMU3 ultratome using glass knives, stained with aqueous uranyl acetate and lead citrate (Reynolds 1963) and viewed with a Philips 400 electron microscope.

RESULTS

Oleo-gumresin ducts are present in primary and secondary phloem (Fig. 1). They are oriented parallel to the longitudinal axis of the stem and anastomose tangentially (Fig. 2) to form an irregular network. Horizontal ducts are present in the rays. The resin droplets that stained dark blue with Sudan black B are abundant in the ducts (Fig. 3). The duct lumen also contains polysaccharides as indicated by the PAS test (Fig. 4).

The duct lumen is bordered by a layer of dense epithelial cells and contains oleo-gumresin (Fig. 5). The subepithelial cells have large vacuoles (Fig. 6). The epithelial cells contain dictyosomes, lipid bodies, mitochondria with dilated cristae, a large nucleus with prominent nucleolus, osmiophilic materials, plastids, and ribosomes (Fig. 7–11). Lipid bodies, mitochondria, osmiophilic materials (resin or its precursor) and plastids are located near the inner tangential wall (the wall facing the duct lumen) of epithelial cells (Fig. 8, 9). Several vesicles (both coated and smooth) are observed in association with dictyosomes in the vicinity of the inner tangential wall (Fig. 11, 12). Sometimes osmiophilic material is present in the dilated dictyosome cisternae...
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Explanation of abbreviations: d = dictyosome; dl = duct lumen; ec = epithelial cell; itw = inner tangential wall; l = lipid; lb = lipid body; m = mitochondrion; mvb = multivesicular body; n = nucleus; nl = nucleolus; os = osmiophilic material; p = plastids; pd = plasmodesmata; pm = plasmamembrane; sec = subepithelial cell; v = vacuole; vs = vesicle.

Fig. 1–5. Transverse (TS) and tangential longitudinal sections (TLS). – 1: TS of secondary phloem showing oleo-gum resin ducts. – 2: TLS of secondary phloem showing anastomosis of two ducts. – 3: TLS of duct showing lipid in the duct lumen, localized by Sudan black B. – 4: TLS of duct showing gum component in the duct lumen stained with PAS (arrows). – 5: TS (electron micrograph) of a duct showing dense epithelial cells. Note presence of osmiophilic material in the duct lumen.

Fig. 6–11. Electron micrographs. – 6: Epithelial cell showing large nucleus with prominent nucleolus, plastids with osmiophilic droplets (arrows) and vacuoles. The subepithelial cell has a large vacuole and peripheral cytoplasm. – 7: Epithelial cell showing large nucleus with prominent nucleolus, lipid bodies, osmiophilic materials, plastids and vacuoles. – 8 & 9: Part of an epithelial cell showing large nucleus with prominent nucleolus. Note dictyosome, lipid bodies, mitochondria, osmiophilic material and plastid near inner tangential wall. – 10: Part of epithelial cell showing lipid bodies, vacuoles, mitochondrion with swollen cristae. – 11: Hypertrophied dictyosomes. Note the smooth and coated vesicles (arrows) associated with dictyosomes; osmiophilic vesicles are present in the apoplast.

Fig. 12–17. Electron micrographs. – 12: Hypertrophied dictyosomes. Note the smooth and coated vesicles (arrow) associated with dictyosomes. Osmiophilic vesicles are present in the apoplast. – 13: Dilated dictyosome cisternae with osmiophilic material. – 14: Plastids without well-developed internal membrane containing osmiophilic droplets (white arrows). Osmiophilic droplets are also seen in the cytoplasm (black arrows). – 15: Multivesicular body containing vesicles with and without osmiophilic material. Note lipid body associated with mvb. – 16: Vesicles and osmiophilic material in the apoplast. – 17: Part of the epithelial cell near the inner tangential wall showing mvb and numerous vesicles.

Fig. 18–23. Electron micrographs. – 18: Osmiophilic material in the apoplast near the inner tangential wall. – 19: Vesicle releasing its contents by exocytosis. Note osmiophilic material deposited along the inner tangential wall (arrows). – 20: Myelin-like bodies near the inner tangential wall (arrow). – 21: Osmiophilic vesicles, myelin-like bodies (arrows) and a disintegrating myelin-like body in the apoplast near the inner tangential wall. – 22: Smooth endoplasmic reticulum releasing osmiophilic material into the apoplast (arrow). – 23: Oblique section of the inner tangential wall showing osmiophilic material on both sides.

Fig. 24–29. Electron micrographs. – 24: Part of the inner tangential wall showing the movement of osmiophilic material into the duct lumen through the loose microfibrils. – 25: Inner tangential wall with lipid body between its loose microfibrils. – 26: Rudimentary plasmodesmata on the inner tangential wall of epithelial cell in young duct (arrows). Note accumulation of osmiophilic material in the duct lumen. – 27: Plasmodesmata between epithelial cell and subepithelial cells. Note the presence of osmiophilic material in the plasmodesmata. – 28: Surface view of the wall between epithelial cell and subepithelial cell showing plasmodesmata. – 29: Part of the inner tangential wall showing sloughing-off of the outer layers into the duct lumen.
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(Fig. 13). The plastids are abundant in the epithelial cells (Fig. 6). They have poorly developed internal membranes and contain osmiophilic droplets (Fig. 6, 7, 14). Osmiophilic materials are also observed in the cytoplasm (Fig. 14). Multivesicular bodies (also called Paramural bodies) containing vesicles with and without osmiophilic material are present in the epithelial cells (Fig. 15, 17). Several electron dense vesicles and osmiophilic materials pile up in the apoplast close to the inner tangential wall (Fig. 16, 18).

Vesicles containing osmiophilic material fuse with the plasmamembrane and release their contents into the apoplast (Fig. 19). Densely stained material comparable to that in the duct lumen is present close to the inner tangential wall (Fig. 19). Myelin-like structures are seen frequently near the inner tangential wall (Fig. 20, 21). They degenerate and form electron dense material (Fig. 21). Smooth endoplasmic reticulum also unload secretory materials into the apoplast near the inner tangential wall (Fig. 22). An oblique section of this wall shows the presence of osmiophilic material on both sides (Fig. 23). The inner tangential wall has loose and dispersed microfibrils (Fig. 24, 25). Osmiophilic materials and sometimes lipid bodies are observed between these loose microfibrils (Fig. 24, 25). Generally plasmodesmatal connections are absent on the inner tangential wall. However, occasionally rudimentary plasmodesmata are observed on the inner tangential wall of young ducts (Fig. 26). Plasmodesmata are abundant between epithelial and neighbouring cells (Fig. 27, 28). The outer layers of the inner tangential wall degenerate and slough off into the duct lumen (Fig. 9, 29).

DISCUSSION

The epithelial cells of the ducts in *Boswellia serrata* synthesize and secrete oleogumresin into the duct lumen. The term oleo-gumresin indicates that the exudate has three components: volatile oil, resin, and gum. It is difficult to differentiate between volatile oil and resin components with the electron microscope as both appear as electron dense material. Both gum and resin are simultaneously formed in the epithelial cells and, therefore, it is assumed that two separate pathways are active in the epithelial cells.

The electron dense material (also called osmiophilic material) in the epithelial cells represents the oleo-resin component of the exudate. Osmiophilic droplets are observed in plastids and dictyosomes which are considered to be the sites of resin synthesis. Plastids without well defined internal membrane structures are known to be a characteristic feature of resin-forming cells (Dell & McComb 1974, 1977; Bhatt 1987). In the glands of *Cannabis sativa* lipid secretion occurs in plastids (Hammond & Mahlberg 1978). According to Bosabalidis and Tsekos (1982a, b) and Heinrich and Schultze (1985) formation of essential oil in *Citrus deliciosa* and *Phellodendron amurense* occurs in plastids. The biosynthesis of oil in leaves of *Laurus nobilis* starts in plastids (Maron & Fahn 1979). It is also suggested that besides plastids and dictyosomes, endoplasmic reticulum, mitochondria and multilamellate structures are possible sites of resin synthesis (Fahn & Evert 1974; Fahn & Benayoun 1976; Bhatt & Shah 1985;
Bhatt 1987; Bhatt & Mohan Ram 1992; Nair 1995). Joel and Fahn (1980b) indicated that various resin fractions are synthesized in different cell components.

The elimination of resin material from the cytoplasm in Boswellia is by granulocrine secretion through the formation of secretory vesicles. The vesicles fuse with the plasmamembrane and release their contents into the apoplast near the inner tangential wall. In this mode of secretion the vesicle membrane becomes part of the plasmalemma and, therefore, considerable membrane recycling occurs. The presence of myelin-like structures along the inner tangential wall indicates this phenomenon. It is also proposed that osmiophilic material may pass through the plasmamembrane as individual molecules against a concentration gradient by a membrane bound molecular pump with ATP supplying energy (eccrine secretion). The presence of mitochondria near the inner tangential wall may provide the necessary energy for transport of the secreted materials. Resin is also eliminated by smooth endoplasmic reticulum. The accumulated osmiophilic material passes through the porous inner tangential wall into the duct lumen. The loose and dispersed microfibrils of this wall facilitate movement of secreted materials across the wall. This mode of elimination of osmiophilic material occurs in Anacardium occidentale (G.M. Nair et al. 1983), Commiphora wightii (Setia et al. 1977; Bhatt 1987), Mangifera indica (Joel & Fahn 1980b; Bhatt & Shah 1985), and Semecarpus anacardium (Bhatt & Mohan Ram 1992). In young ducts the secreted material may pass through the rudimentary plasmodesmata on the inner tangential wall. Fahn and Evert (1974) reported that in Rhus glabra the rudimentary plasmodesmata provide channels for transport of secretory material through the inner tangential wall.

The gum component in the secretion may be formed by two methods. The gum polysaccharide is synthesized in the dictyosomes and deposited in the apoplast through several smooth vesicles by exocytosis. It is then transported into the duct lumen through the porous inner tangential wall. The involvement of dictyosomes in the synthesis and transport of polysaccharide is well established in plants (Mollenhauer & Morre 1966; Northcote & Pickett-Heaps 1966; Morre et al. 1967; Fahn & Rachmilevitz 1970; Gunning & Steer 1975; Rachmilevitz & Fahn 1975; Fahn 1979; Fahn & Benouaiche 1979; Mauseth 1980; Schnepp & Christ 1980; Rougier 1981; Trachtenberg & Fahn 1981; Gedalovich & Fahn 1985; Sedgley & Blessing 1985; Bhatt 1987).

Alternatively the gum is also derived from the outer layers of inner tangential walls. Portions of degenerating cell wall in the duct lumen and sloughing-off of the outer layers of the inner tangential wall support this view. The inner tangential wall is replenished from inside by the activity of dictyosomes which synthesize and deposit polysaccharide for the formation of new wall layers. A similar mode of formation of gum has been observed in some other species (Fahn & Evert 1974; Setia et al. 1977; G.M. Nair et al. 1983; M.N.B. Nair et al. 1983; Bhatt & Shah 1985; Bhatt 1987; Bhatt & Mohan Ram 1992; Venkaiah 1990, 1992; Nair 1995).

The coated vesicles associated with dictyosomes are known to be involved in oil body development (Galatis et al. 1978), transfer of carbohydrate to cell wall (Hepler & Newcomb 1967; Cronshaw & Esau 1968; Fowke et al. 1975; Bhatt 1987), and primary and secondary wall formation (Ryser 1979). Therefore, it is plausible that the
coated vesicles in the epithelial cells might be associated with formation of volatile oil and/or replenishment of the inner tangential wall. The numerous plasmodesmata between epithelial and subepithelial cells indicate that there is movement of substances from the surrounding cells to the epithelial cells as proposed earlier (Tucker 1982; Gunning & Overall 1983; Esau & Thorsch 1985; Bhatt 1987).

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