ULTRASTRUCTURE AND HISTOCHEMISTRY OF THE WATERMELON STIGMA

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SUMMARY
The ultrastructure and histochemistry of the watermelon stigma were followed from 6 days before flower opening to anthesis. Starch and lipid were present in the immature papilla cells but had largely disappeared from the cells by anthesis, when carbohydrate and lipid were present in the stigma secretion external to the cell wall. The mature papilla cells had simple wall thickenings and were transfer cells. The wall thickenings were associated with dictyosomes and secretory vesicles. Other characteristics indicating a secretory function for the papilla cells included plastid–ER complexes and close associations between ER and secretory vesicles at the plasmalemma. Granulocrine secretion involving dictyosomes and ER is suggested for the carbohydrate component of the secretion.

INTRODUCTION
The surface of the angiosperm stigma is specialized for pollen hydration and germination by the presence of a secretion which may be copious ('wet' type) or barely present ('dry' type) (Heslop-Harrison & Shivanna, 1977). In 'dry' type stigmas the secretion consists of protein and lipid (Mattson, Knox, Heslop-Harrison & Heslop-Harrison, 1974). The secretion of 'wet' type stigmas may consist largely of lipid (Konar & Linskens, 1966; Martin, 1969; Baker, Baker & Opler, 1973; Dumas, 1974b; Lord & Webster, 1979), of carbohydrate (Loewus & Labarca, 1973; Portnoi & Horovitz, 1977; Kristen, Biedermann, Liebezit & Dawson, 1979), or of both (Sedgley & Buttrose, 1978; Sedgley & Scholefield, 1981). The stigma papilla cells are generally considered to produce the secretion, but opinions differ as to which organelles are most actively involved. Kroh (1967), Dumas (1974b) and Kristen (1977) reported that the endoplasmic reticulum (ER) was responsible for the secretion, whereas Vasil'ev (1970) and Shiraishi, Matsumoto & Shigemasu (1976) considered that the dictyosomes were most active. In this paper the ultrastructure of the watermelon stigma and its relationship with the secretion are described. Both the dictyosomes and ER are considered to be involved in the production of stigma secretion.

MATERIALS AND METHODS
Plant material
Watermelon (Citrullus lanatus (Thunb.) Matsum & Nakai, cv. 'Early Yates') plants were grown in a growth cabinet with a 25 °C day, 20 °C night, 14 h photoperiod and a photon flux density of 640 μE m⁻² s⁻¹ (400–700 nm). Unpollinated stigma tissue was taken from female
flowers from 6 days before opening to anthesis. The age of unopened flowers was estimated from longitudinal measurements of the inferior ovary of flowers that were allowed to open. Ovary length doubled between 4 days before opening and anthesis.

**Scanning electron microscopy**

Fresh uncoated tissue was mounted on spiked stubs and observed in a Philips SEM 500 at 6 kV.

**Thin-section electron microscopy**

Stigma tissue was fixed either in 3 % glutaraldehyde in 0.025 M phosphate buffer, pH 7, for 4 h followed by postfixation in 1 % osmium tetroxide in the same buffer or in 2 % aqueous potassium permanganate for 2 h with no postfixation in osmium. Tissue was dehydrated in an ethanol series, through propylene oxide, and embedded in Araldite. Sections mounted on grids were stained with uranyl acetate and lead citrate and viewed with a Philips EM400 at 80 kV.

**Light microscopy and histochemistry**

Glutaraldehyde-fixed tissue was embedded in glycol methacrylate (GMA). Sections were cut at 2.25 μm and stained with periodic acid-Schiff’s reagent (PAS) or PAS followed by toluidine blue O (Feder & O’Brien, 1968). Araldite-embedded tissue was sectioned at 1 μm and stained with Sudan black (Bronner, 1975).

**RESULTS**

The watermelon stigma consisted of 3 grooved lobes (Fig. 1). The papilla cells were grouped together to form multicellular structures (Fig. 2). Beneath the papillae were thin-walled parenchyma tissue with no intercellular substance and also transmitting tissue, with PAS-positive walls and intercellular substance, which extended down the solid style.

Starch was present in the papilla cells of the immature stigma with maximum accumulation at one day prior to anthesis (Fig. 3). Very little starch was present in the mature stigma (Fig. 4). The cell wall was PAS-positive and thickenings of the wall

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Fig. 1. Scanning electron micrograph of watermelon stigma 2 days before anthesis. g, groove; l, lobe. ×60.

Fig. 2. Light micrograph of longitudinal section of watermelon stigma at anthesis, embedded in GMA and stained with PAS and toluidine blue O. pt, parenchyma tissue; tt, transmitting tissue; arrowheads show grouped papilla cells. ×30.

Fig. 3. Light micrograph of longitudinal section of watermelon stigma papilla cells one day before anthesis, embedded in GMA and stained with PAS. pc, papilla cell; s, starch grain; se, secretion. ×400.

Fig. 4. Light micrograph of longitudinal section of watermelon stigma papilla cells at anthesis, embedded in GMA and stained with PAS. pc, papilla cell; s, starch grain; w, wall thickenings. ×200.

Fig. 5. Electron micrograph of longitudinal section of watermelon stigma papilla cells 6 days before anthesis, fixed with glutaraldehyde. er, ER; l, lipid; pd, plasmodesmata; v, vesicle; va, vacuole. ×16,500.

Fig. 6. Light micrograph of longitudinal section of watermelon stigma papilla cells at anthesis embedded in araldite and stained with Sudan black. l, lipid; pc, papilla cell; se, secretion; w, wall thickenings. ×600.
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were present at anthesis (Fig. 4) but not at earlier stages (Fig. 3). Processing for GMA-embedding removed the secretion from the exposed papillae (Fig. 4). However, the PAS-positive nature of the secretion was visible in the grooves of the stigma where the papillae were close together (Fig. 3). Lipid droplets were present in the papilla cells of the immature stigma (Fig. 5). Their presence was confirmed by staining with Sudan black. In the mature stigma lipid was present in the secretion (Figs. 6, 7) but very little was present in the cells (Fig. 6). In some cases the lipid appeared to have been partially extracted during processing (Fig. 10). The secretion had a fibrillar structure (Figs. 7, 9, 10, 14).

The cytoplasm of the immature papilla cells was very dense with abundant ribosomes (Figs. 5, 9). Plasmodesmata were present in the walls and vesicles were observed in association with the cell walls (Fig. 5). In the very early stages there were small vacuoles but no large central vacuole as in the mature papilla cell (Fig. 7). The large and small vacuoles of the mature cells contained membranous and granular inclusions. The wall thickenings were associated with dictyosomes and vesicles (Fig. 8). The nucleus of the papilla cell was lobed (Fig. 10).

Plastids were present in the papilla cells. They contained starch in the immature cells (Fig. 9) and were irregular in outline in the mature cells (Figs. 7, 10, 11). They often occurred in close association with each other (Fig. 11) or with other organelles including mitochondria (Fig. 10), ER (Figs. 10, 11), and dictyosomes (Figs. 9, 10). The plastids had relatively simple membrane systems and in the mature stigma contained crystals, plastoglobuli and bundles of tubules (Fig. 11).

Dictyosomes were common in both the immature (Fig. 9) and mature (Figs. 8, 10, 12, 13, 15) papilla cells. With glutaraldehyde fixation there was a progressive increase in the staining intensity of the cisternae from the forming face to the maturing face (Fig. 12). The vesicles had fibrillar contents (Figs. 8, 12) and dilated areas were sometimes present in the middle of a cisterna (Fig. 12). With potassium permanganate fixation the dictyosomes had a typical curved appearance and the contents of the vesicles appeared to have been extracted during processing (Figs. 13, 15). Vesicles were observed in association with the plasmalemma around the wall thickenings (Fig. 8) and also on the non-thickened areas of cell wall (Fig. 15). In some cases a core of dense fibrillar material developed in the vesicle (Fig. 14). ER was present during all stages of stigma development (Figs. 5, 9, 10, 11, 15). It was closely associated with the plasmalemma and the vesicles (Figs. 5, 15). Both smooth and rough ER were observed in association with the plasmalemma (Figs. 5, 9, 10). ER was also closely associated with plastids (Figs. 11).

Fig. 7. Electron micrograph of transverse section of watermelon stigma papilla cell at anthesis, fixed in glutaraldehyde. i, inclusion in vacuole; l, lipid; pl, plastid; se, secretion; va, vacuole; w, wall thickening. ×7000.

Fig. 8. Electron micrograph of watermelon stigma papilla cell at anthesis showing wall thickening, fixed in glutaraldehyde. cte, cell wall; d, dictyosome; v, vesicles; w, wall thickening. ×31000.
DISCUSSION

Thickenings of the cellulose cell wall are characteristic of transfer cells and occur in situations where there is a high flux across the plasmalemma (Gunning & Pate, 1974). Copious secretion occurs in response to pollination of the watermelon stigma (Sedgley & Scholefield, 1981). The wall thickenings of the papilla cells are associated with dictyosomes and vesicles and probably increase the surface area across which the secretion can pass. Transfer cells have not previously been reported in stigmas, although they occur in the style and ovary of some species (Rosen & Thomas, 1970; Vasil'ev, 1970; Gunning & Pate, 1974; Johnson, Wilcoxson & Frosheiser, 1975).

The secretion of the watermelon stigma consists of carbohydrate, lipid and water with protein confined to the outer surface (Sedgley & Scholefield, 1981). The immature papilla cells contain starch and lipid. The mature papilla cells are very low in these reserves but have carbohydrate- and lipid-containing secretion external to the cell wall. The starch and lipid of the immature cells are probably used to manufacture the secretion.

The fibrillar areas of the secretion correspond to PAS-positive material (Sedgley & Scholefield, 1981). Vesicles produced by the dictyosomes contain fibrillar material of similar appearance to that in the secretion. In general, carbohydrate-secreting glands show granulocrine secretion via dictyosome vesicles (Schneppf, 1974). However, ER is closely associated with the vesicles at the plasmalemma and it is suggested that both the dictyosomes and the ER are associated with granulocrine carbohydrate secretion in the watermelon stigma. Kristen et al. (1979) reported that granulocrine secretion via ER-vesicles produced the carbohydrate-rich stigma secretion in *Aptenia cordifolia*. In this tissue the dictyosomes were weakly developed (Kristen, 1977). However, dictyosome vesicles were active in the production of the largely polysaccharide stigma secretion in *Verbascum phlomoides* (Dumas, 1974a). Both ER and dictyosomes have been implicated in protein secretion (Hanke, 1977) and the protein in the outer surface of the watermelon stigma secretion may also be produced via these organelles. The extracted vesicles in the potassium permanganate-fixed tissue may have contained polysaccharide and protein in vitro as this fixative is a strong oxidizing agent.

The passage of lipid between the cytoplasm and the secretion was not observed in this study. However, both Dumas et al. (1978) and Sedgley (1979) have published micrographs showing lipid droplets in the cellulose cell wall of stigma papillae. Kroh

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**Fig. 9.** Electron micrograph of watermelon stigma papilla cell 2 days before anthesis, fixed in glutaraldehyde. *d*, dictyosome; *er*, ER; *l*, lipid; *pl*, plastid; *s*, starch; *se*, secretion. × 12,500.

**Fig. 10.** Electron micrograph of watermelon stigma papilla cell at anthesis, fixed with glutaraldehyde. *d*, dictyosome; *er*, ER; *l*, lipid; *m*, mitochondrion; *n*, nucleus; *pl*, plastid; *se*, secretion. × 17,500.

**Fig. 11.** Electron micrograph of watermelon stigma papilla cell at anthesis showing a group of plastids, fixed in glutaraldehyde. *c*, crystal; *er*, ER; *pg*, plastoglobuli; *pl*, plastid; *t*, bundle of tubules. × 20,000.
Fig. 12. Electron micrograph of watermelon stigma papilla cell at anthesis showing dictyosome, fixed with glutaraldehyde. ff, forming face; mf, maturing face; v, vesicle. x 49000.

Fig. 13. Electron micrograph of watermelon stigma papilla cell at anthesis showing dictyosomes, fixed with potassium permanganate. d, dictyosome; v, vesicle. x 16000.

Fig. 14. Electron micrograph of watermelon stigma papilla cell at anthesis showing vesicles, fixed with glutaraldehyde. cw, cell wall; pl, plastid; se, secretion; v, vesicle. x 17500.

Fig. 15. Electron micrograph of watermelon stigma papilla cell at anthesis showing ER and vesicles, fixed with potassium permanganate. d, dictyosome; er, ER; v, vesicle; pm, plasmalemma.
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(1967) reported granulocrine secretion via ER-vesicles of the largely lipidic stigma exudate of *Petunia hybrida*. Plastids and ER and plastids sheathed by ER are typical of lipid-secreting glands (Schnepf, 1974). Such organelles and configurations are present in the watermelon stigma papilla cells and also in those of *Forsythia intermedia*, *Verbascum phlomoides* (Dumas, 1974a) and *Lycopersicum peruvianum* (Dumas et al. 1978). The plastids of the mature watermelon stigma are chromoplasts because crystals, plastoglobuli and bundles of tubules all contain carotenoids (Frey-Wyssling & Kreutzer, 1958).

The stigma secretions which have been described to date reportedly contain largely carbohydrates, lipids and proteins in varying amounts. The ultrastructure of the stigma papilla cells suggests secretory activity and the variations which have been reported probably reflect differences in composition of the secretory product.

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REFERENCES


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