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STIGMA AND STIGMATOID TISSUE OF LYCOPERSICON ESCULENTUM MIL.

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SUMMARY

The development and structure of the stigma and stigmatoid tissue of *Lycopersicon esculentum* were studied by means of electron and light microscopy. The stigma cell originate from several periclinal divisions of the epidermal and subepidermal cells of the top of the style. The stigmatoid tissue stems from divisions of the ventral epidermis cells of the carpels and fills the centre of the style. This tissue is the extension of the stigma cells. In ovular direction the stigmatoid tissue is divided into definite strands. During differentiation the cells of stigma and stigmatoid tissue change structurally, develop more ER and lomasomes. Ultimately the intercellular substances between the cells of the stigma en stigmatoid tissue contain mainly lipids, but also carbohydrates and proteins. The ER is suggested to be active in lipid production or a precursor of lipids probably emerges from the cells of the described tissue and forms (outside of them) intercellular substances. The fatty acids on the stigma surface are also present in the pollenkitt.

1. INTRODUCTION

Stigma and stigmatoid tissue are frequently studied in various aspects. This is manifested by the following recent works: KNOX (1972), KROES (1973), SASSEN (1974), KROH & HELSPER (1974), MATSON et al. (1974), BELL & HICKS (1976), CRESTI et al. (1976, 1982), KADEJ et al. (1977, 1980), DUMAS et al. (1978), STEAD et al. (1980), J. & Y. HESLOP-HARRISON (1980, 1981, 1982), HERRERO & DICKINSON (1979), TAKAHASHI & TAKEDA (1981), SHIVANNA & SASTRI (1981), WILMS (1980), PACINI (1981) and CIAMPOLINI et al. (1983). Because the stigma plays an important role in the process of hybridization in acceptance of pollen, most authors have studied the structure and function of the pistil, especially of the stigma and stigmatoid tissue in relation to pollination. A general view of structure and function, even in wet stigmas, cannot yet be given and further study of the stigma is needed. On account of its economic significance the flower of *Lycopersicon esculentum* has been described in detail on light microscopical level (SMITH 1935; RICK 1980). These elementary anatomical studies of the stigma and style of *Lycopersicon esculentum*, which seem to have a genetic uniformity (RICK 1980) and a simple perspicuous anatomy, are mainly focussed on developmental aspects. The present study will also be related to the earlier study of the transmitting tissue of an incompatible and wild type of tomato *Lycopersicon peruvianum* Mill. (CRESTI et al. 1976).

2. MATERIAL AND METHODS

Self-compatible plants of *Lycopersicon esculentum* Mill cv. Bonny best were grown in a greenhouse. Styles of 0.1 to 9.0 mm were collected for light and electron microscopy. Paraffin sections were stained with safranin fast green (JENSEN 1962). For electron microscopy tthe stigmas and styles were prepared according to CRESTI et al. (1976), for scanning microscopy according to WILMS (1980). To detect several structural components in fresh sections auramine-O was used for lipid substances; the periodic acid-Schiff (PAS) reaction for carbohydrates and Coomassie blue for proteins (JENSEN 1962; HESLOP-HARRISON & HESLOP-HARRISON 1980).

For gaschromatography about one hundred and fifty tips of mature styles before and after pollination as well as the pollen from about fifty stigmas were used for lipid extraction in hexane. The method was followed to collect the intercellular substance and pollenkitt.

The material was dried, dissolved in 250 μl hexane and then saponified in 0.3 N KOH in methanol at room temperature during five minutes. After evaporation of the hexane layer the samples were dissolved in 50 μl hexane and about 1 μl was injected on a Silar-10 Cp-coated support at 220 °C.

For thin layer chromatography the monosaccharides of the stigmatic fluid of ten stigmas were separated with ethyl-acetate, pyridine, water, acetic acid and propionic acid (5:5:1:0,5:0,5) on silical gel (Merck) using glucose, fractose, raffinose and trehalose as references. The detection of monosaccharides was done with dichloric fluoresceine according to KLAUS & RIPPHAHA (1982).

3. RESULTS

3.1. Development of stigma and stigmatoid cells

The duration of flower development in its different parts was in accordance with the data for *Lycopersicon peruvianum* of PACINI & SARFATTI (1978). Ontogenetically the pistil develops as the last part of the flower and differentiates into the ovary, style and stigma. The pistil primordia, composed of two or three connate carpels, exist in the early stage of the development of meristematic cells only. The ovary develops first, then the style is initiated by the incipient growth of the cells in the top region. During the early stages of development, furrows between the carpels remain present in the stigmatic surface (*fig. 1*).

Till a length of about 0.1–0.2 mm the growth of the stigma is mainly due to periclinal divisions of the epidermal and subepidermal cells of the top of the style resulting in regular cell rows (*fig. 4*). These cells also undergo anticlinal divisions extending the terminal part of the style – the stigma. The most external cell layer differentiates into papillae which are of moderate length (*fig. 3*). Initially the papillar cells possess a widened base covering the elongated stigma cells situated below (*fig. 2*). At a length of about 2 mm the papillar cells gradually reduce their contact.

The stigmatoid tissue is the result of several periclinal divisions starting mainly

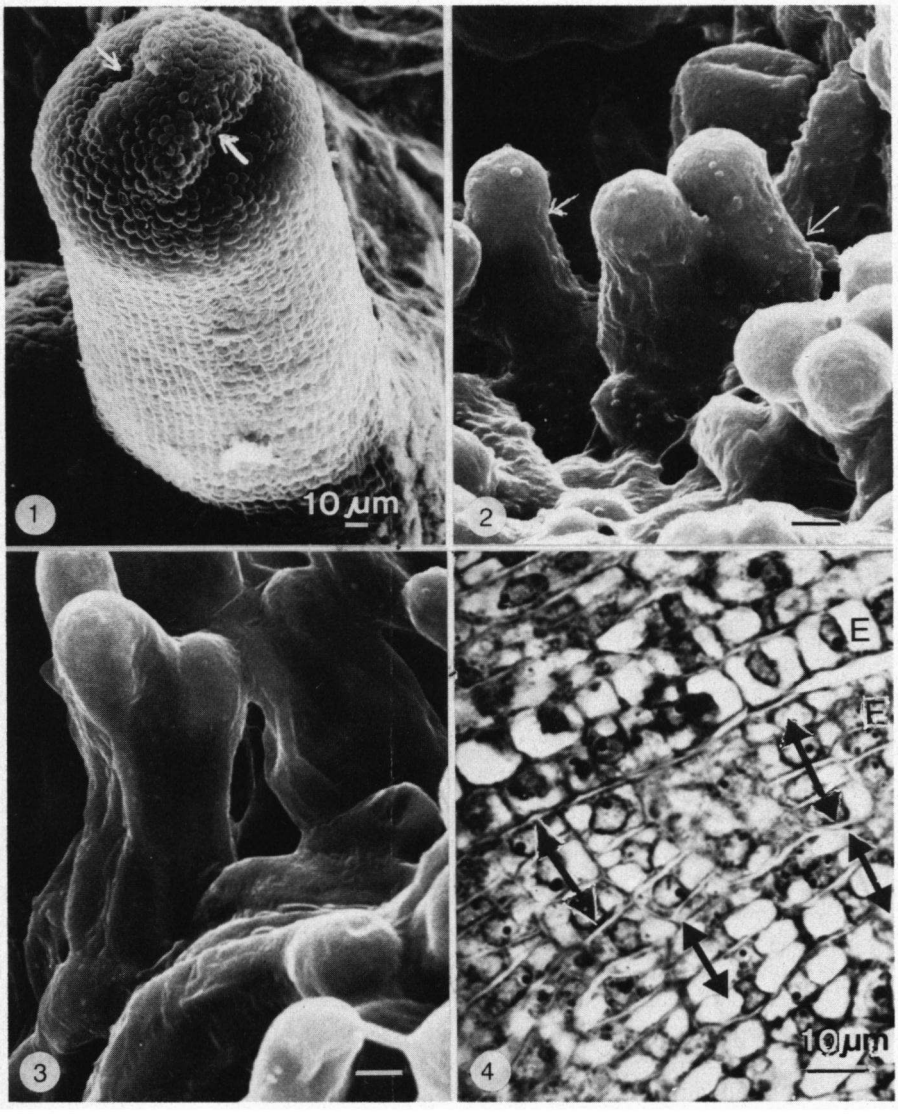

Plate I. Line in figs. 2, 3 indicates 1 μm.

Fig. 1. Stigmatic surface of a 0.1 mm stigma showing the end lobes, furrow (arrows), and undifferentiated cells. × 270.

Fig. 2. Developing stigmatic cells from a widened base. Double ended arrow indicates a widened base. × 6000.

Fig. 3. Differentiation of papillar cells. Elongation of papillae does not occur independently. × 6000.

Fig. 4. Origin of rows of stigmated cells by periclinal dividing epidermal (E) and subepidermal cells. Double ended arrows indicate dividing direction. × 900.

in the ventral epidermis cells of the growing carpels (*fig. 4*). These periclinal divisions are initiated below the stigma and progress downward to the base of the style. This causes a further extension of the stigma cells. Soon after the divisions, the cells elongate and form a homogeneous tissue in the central part of the style. At 2/3 the length of the style toward the ovary, the compact strand of stigmatoid tissue is gradually divided in three or two groups of single strands depending on the number of carpels (*fig. 5*).

3.2. Ultrastructure of stigma and stigmatoid cells

The epidermal cells of the pistil primordium at a stylar length of 0.5 mm show a meristematic character. At the earliest stage a large nucleus, proplastids, abundant ribosomes and short strands of endoplasmatic reticulum (ER), lipid droplets, and small vacuoles sometimes containing electron-dense material, are present (*fig. 6*). Subsequently the plasmamembrane shows large undulations enclosing lomasomes (*fig. 7*). In the basal part of stigma cells only a small middle lamella is visible (*fig. 7*).

At a stylar length of 1 mm, the most external cells of the stigma, derivatives of the prior epidermis, differentiate into papillar cells (*fig. 2*). Their vacuoles with lipid droplets enlarge, the starch formation and ER (mainly smooth ER) elongation occur and thin cell walls are present (*fig. 8*). In a maturing stigma the papillar cells develop large vacuoles in their extensions.

During the further differentiation of the stigma and stigmatoid tissue the thickness of the walls remains constant. Between the cells of both tissues the intercellular cavities increase in size and an electron-dense substance appears. The quantity of the electron-dense material increases gradually and a granular zone becomes visible in the middle of the intercellular material (*fig. 9*). From this moment the stigmatoid tissue and stigma cells gradually loose contact. In styles of about 5 mm the formation of electron-transparent globular structures becomes apparent (*fig. 10*). Subsequently fusion of these electron transparent globules occurs (*fig. 11*).

The very young stigma cells are covered with an uniform cuticle (*figs. 6, 8*). At a stylar length from 2.5 mm the top shows a negative auramine-O staining (*fig. 12*). After the disappearance of these lipid substances, the stigma surface becomes a loose arrangement and papillar structures arise. From that moment an exudate, originating from the intercellular areas of the stigma and stigmatoid tissue, is present and increases in quantity.

3.3. Histochemistry and chromatography of the intercellular substance

Sections of the stigma and stigmatoid tissue stained with osmium tetroxyde or with sudan black show a positive reaction in the intercellular substance from very early stages of about 1 mm stylar length. This reaction indicates the presence of lipid substances. Staining with Nile blue reveals a chemical difference between the substance in the intercellular space of a mature style and the droplets on the stigma surface outside. In the stigmatoid tissue a blue colour indicates a

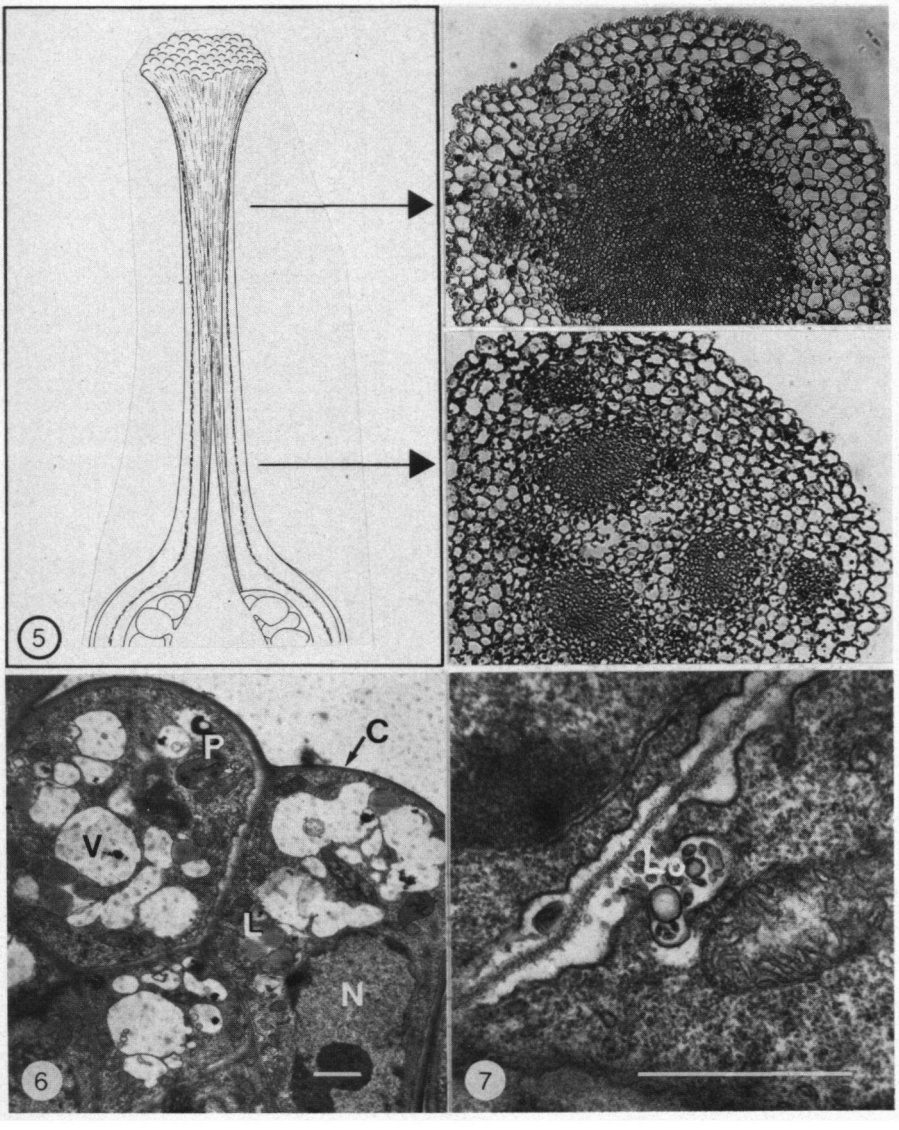

Plate II. Line in the figures indicates 1 μm.

Fig. 5. Schematic representation and cross sections of the developing stigmatoid tissue. st = stigmatoid tissue; vb = vascular bundle. × 250.

Fig. 6. Stigma cell of a 0.5 mm long style. C = cuticle; L = lipid droplet; N = nucleus; P = plastid; V = vacuole. × 5400.

Fig. 7. Undulating plasma membrane and lomasomes (Lo) of a young stigma cell. Note the small middle lamella. × 29,700.

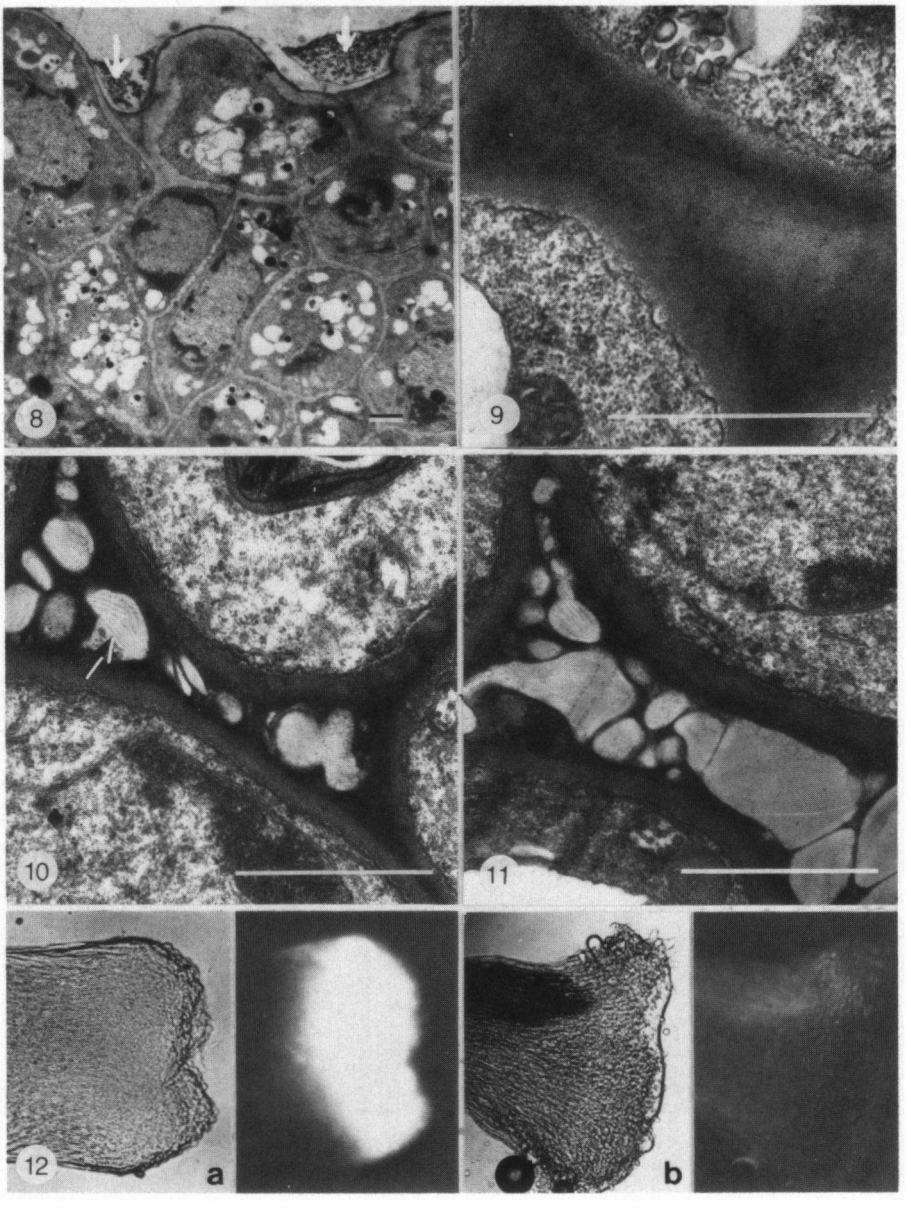

Plate III. Line in the figures indicates 1 μm.

Fig. 8. Survey of stigma cells at a stylar length of 1 mm. At the papillar cells electron-dense material is present (arrow). × 3600.

Fig. 9. Intercellular substances in stigmatoid tissue at a stylar length of 1 mm. Containing a granular material. × 35,100.

basic lipophylic nature. Pollenkitt reacts in the same way as the droplets on the surface, which show a red colour, indicating an acid lipophylic nature. Gaschromatographical analysis of the stigmatic exudate before and after pollination shows no differences. The exudate consists of palmitic, stearic, and oleic acid as components, while lauric, linoleic and behemic acid are also present. Pollenkitt contains palmitic, stearic and oleic acid, but also myristic acid. Lauric, linoleic and behemic acid seem to be absent.

The intercellular substance shows positive staining with PAS. Thin layer chromatography reveals the presence of sucrose, fructose, glucose and probably raffinose. Staining with Coomassie blue on fresh sections shows the presence of proteins.

4. DISCUSSION

According to HESLOP-HARRISON & SHIVANNA (1977) the stigma of *Lycopersicon esculentum* belongs to the group of wet stigmas with a receptive surface with relatively small papillae. Cells of the stigma and stigmatoid tissue are formed and differentiated in the same way. The stigma cells originate from several periclinal divisions of the epidermal and subepidermal cells on the top of the style; the stigmatoid tissue, according to VASIL & JOHRI (1964), is the result of similar divisions of the ventral epidermis of the carpels and forms the central part of the style. These divisions are initiated in the stigma and progress downward to the base of the style. Thus the stigma cells are directly continuous with the stigmatoid tissue forming one path leading from the stigma through the style to the ovary. In agreement with ESAU (1960) and KONAR & LINSKENS (1966), but in contrast with CRESTI et al. (1976) and DUMAS et al. (1978) for *L. peruvianum*, it therefore is convenient to describe this tissue as stigmatoid. KONAR & LINSKENS (1966) and SEDGLEY & BUTTROSE (1978) also have not observed any differentiation between the cells of the stigma and stigmatoid or transmitting tissue. In ovular direction the stigmatoid tissue becomes divided into definite strands. The number of strands is related to the number of carpels forming the pistil, which is comparable to the data for cotton (JENSEN & FISHER 1969).

In a young style the cells of the stigma and stigmatoid tissue show a meristematic character. The observed retraction of the plasmamembrane is comparable to the same process in the transmitting tissue of *L. peruvianum* (CRESTI et al. 1976). The ultrastructure of the stigmatoid cells and cells of the transmitting tissue between the two species are nearly comparable, only plasmodesmal con-

Fig. 10. Globular electron-transparent structures between the stigmatoid cells showing electron-dense strands (arrow). Stylar length 5 mm. × 27,000.

Fig. 11. Fusion of globular electron transparent structures inside the intercellular substances. × 27,000.

Fig. 12. Auramine-O stained stigmata showing the lipid substances present on a style of 1 mm length (a), absent on a style of 2.5 mm length (b). × 115.

tact in the stigmatoid tissue of *L. esculentum* exists in all directions, whereas in *L. peruvianum* plasmodesmal contact is only present in the short transverse cell walls.

In the developing stigmatoid tissue the increase of ER, mainly SER, is probably related to the production of the high quantity of lipids. The intercellular substance in the stigmatoid tissue of *L. esculentum* is composed mainly of lipids and contains less carbohydrates and some proteins. In the transmitting tissue of *L. peruvianum* (CRESTI et al. 1976) lipids seem to be absent, although the intercellular substance is very osmiophilic. In the exudate of *L. esculentum* the scala of lipid acids analysed is nearly the same as in the pollenkitt, which is confirmed by the Nile blue reaction. This means the fatty acid part is qualitatively common in pollenkitt and exudate. The difference in the Nile blue reaction between the stigmatoid intercellular substance and the exudate outside indicates a transition of the lipids from a basic to an acid nature, probably by oxydation.

The nature of the electron-transparent zones in the intercellular substance of the stigmatoid tissue, which is also present in the transmitting tissue of *L. esculentum* (CRESTI et al. 1976), is unknown. The composition of the intercellular substances seems to remain unchanged throughout the differentiation of the cells of the stigma and stigmatoid tissue. Because of the relatively sudden disappearance of the lipid substances not only disruption of the cuticle but also an oxydation or a mixture of both can be the cause of change.

Compared with other studies on stigma and stigmatoid tissue in *L. esculentum* each plant shows its own type of differentiation. A general extension to other organisms or an elucidation of the function will need further studies.

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