# STIGMA OF *DATURA STRAMONIUM* L. (SOLANACEAE): HISTOGENESIS, MORPHOLOGY AND DEVELOPMENTAL ANATOMY

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## Abstract

The structure and histogenesis of the stigma of *Datura stramonium* was investigated by light and scanning electron microscopy to determine the developmental origin of the transmitting tissue and "wet stigma". Four developmental stages were defined on the basis of bud length. Stage one, the histogenesis stage, was comprised of buds 2-15 mm in length. Stage two, signified by the growth and development of the stigma was comprised of buds 15-40 mm long. Stage three, with buds of 40-60 mm in length, represented a receptive and mature stigma. Stage four started with self pollination (occurs prior to anthesis) and ended with stigma senescence. A developing stigma showed a bilobed, papillated surface, covered with sticky secretion. The stigma consisted of two distinct zones; 1) Superficial zone; formed by papillae and 5-8 layers of secretory cells immediately beneath them. This zone was subjected to a gradual lysis in the ontogeny of the stigma. 2) Internal zone; formed by the transmitting tissue of the style. This zone remained intact throughout the development. Transmitting tissue and stigma both originated from the epidermis of the carpels. Similarities in structural organization of the stigma of *Datura* to those reported in *Solanum tuberosum* were considerable.

## Introduction

*Datura stramonium* is a member of the *Solanaceae* that grows wild in Iran. It is an annual plant with white flowers, a pentamerous trumpet shaped corolla, five stamens and two fused carpels. All parts of *Datura* contain poisonous alkaloids like hyoscyamine, scopolamine, and atropine. Because of the pharmaceutical importance, it has been studied in detail by biochemists [10,12,14]. In addition, *Datura* is a favorable plant for genetic research because of its reduced number of chromosomes (2n=24) [9]. Despite the considerable work published on different aspects of *Datura*'s biochemistry and genetics, there is little information on its reproductive biology. Satina [15], described the stratified structure of the reproductive meristem and initiation and development of diverse floral organs in *Datura*. She also reported the epidermal origin of the stigma and transmitting tissue in the style of the plant. However, she did not provide a clear description of the stigma histogenesis and its structure.

The stigma is considered of great importance in the

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pollination-fertilization process, because it is the first place that is in contact with pollen grains. Thus its structure and histochemical studies are an important part of the general investigation in reproductive biology. In the past 30 years many of the economically important plants of the *Solanaceae* were the subject of detailed studies in this field [1,3,7,8,11,13].

The objective of the present study was to answer the following questions: 1) What is the developmental origin of the stigma and transmitting tissue of *Datura*, e.g., is this tissue strictly epidermal in origin? 2) What is the developmental and structural basis for the "wet type" stigma as described by Heslop-Harrison and Shivanna [5] for *Datura*? They categorized *Datura*'s stigma as Group IV, a wet non-papillate one. This is in contrast to several other *Solanaceae* which they listed as Group III, a wet papillate stigma.

## Materials and Methods

During the months of August and November, the flowers were collected from wild plants growing well in the fine sandy loam soil at the border of Ajy river. This river is in the northern part of Tabriz, Iran. These flowers were sampled at various developmental stages including anthesis with the samples grouped in 25 categories based on the length of the floral buds (Fig. 1).

The whole buds at less than 10 mm long and the stigma of the buds between 10 and 100 mm long were fixed in neutralized formalin (10%) for 24 h at room temperature (RT). Samples were dehydrated, cleared in an alcohol-xylene series, and then embedded in paraffin blocks. These blocks were then cut at 5-7 µm and subjected to 1) feulgen-aniline blue staining, [6] for histologic studies and 2) periodic acid Schiff reagent (PAS) [4] for revealing the cell walls and demonstration of cell contours. Some samples were fixed in a mixture of 2% formaldehyde and 3% glutaraldehyde in 0.2 M cacodylate buffer (pH=7.2) for 24 h. Then were rinsed in buffer and post fixed in Osmium tetroxide (2% in cacodylate buffer) for 2 h, followed by buffer rinsing and dehydration with ethanol series. Specimens were embedded in spur medium. Semi-thin cross sections (2-3 µm) were cut on a LKB ultramicrotome. Sections were then stained with Sudan black B [4]. Slides were examined and photographed using a Zeiss photomicroscope.

For Scanning Electron Microscopy (SEM) flower buds ranging from 10 mm to 65 mm in length were

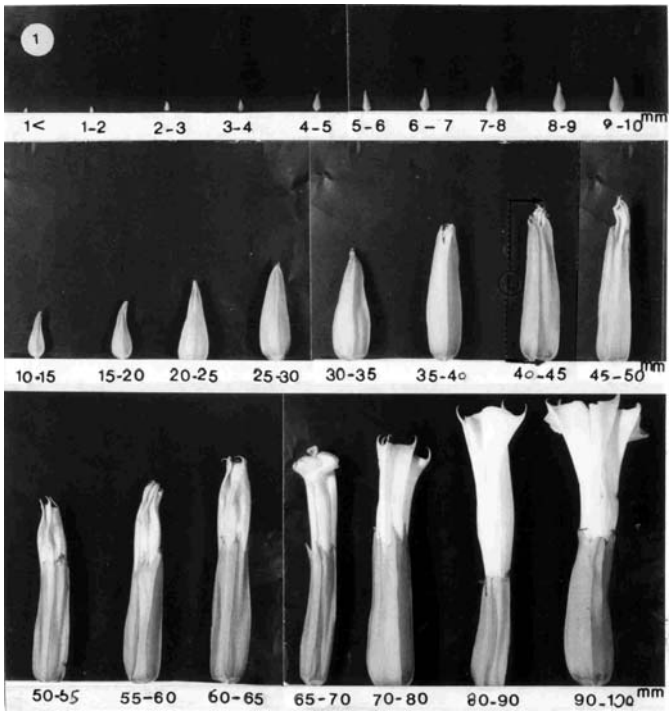



**Figure 1.** 25 different stages were determined arbitrarily in *Datura stramonium* ontogeny based on the length (L) of the buds.

sampled. Stigmas were fixed in 2.5% glutaraldehyde for 90 min at RT. After washing with 0.2 M phosphate buffer (pH=7.3), samples were post fixed in Osmium tetroxide (1% in phosphate buffer) for one h. Samples then were dehydrated in an ethanol series and mounted on a specimen stub, secured with glue, coated with gold

(2-4 min) and viewed in a Hitachi, HHS SEM at 25 KV.

## Results

### Morphology

The stigma of *Datura* was a lateral type unlike many other plants of the *Solanaceae*. In the lateral type stigma, the papillae totally cover the ventral faces of the stigma [2]. In the stigma of *Datura stramonium*, papillae extended some way down the style, following the junction line of the two carpels in the ventral faces. The developing stigma was a flat, bilaterally symmetrical bilobed structure, separated by a slight central depression (Fig. 2). The stigma presented two different appearances in dorsal and ventral faces (Fig. 3). The dorsal surface was an extension of the terminal part of the two carpels coming in contact with each other and forming a ventral face. The dorsal face was flattened which appeared as a cap-shaped can. The ventral surface was heart-shaped and convex.

The surface of the stigma was covered with large number of unicellular papillae. They appeared in the early stages in the form of densely bubbles at the tip and around the central depression of the young stigma (Fig. 4). Gradually, the stigmatic papillae extended to the dorsal and particularly to the ventral faces following the junction line of the carpels, thus extending the stigmatic surface. As development proceeded, the papillae expanded in length often appearing in the form of thumb with an enlarged base (Fig. 5). The most obvious feature of stigma development was the progressive detachment of papillae from the stigma surface. Furthermore, the secretory cells beneath the papillae were involved in detachment processes, causing the formation of surface pits and grooves at late stages of the development (Fig. 6). Scanning electron microscopy revealed the amorphous and sticky nature of the secretion in the mature stigma (Fig. 7).

### Histogenesis

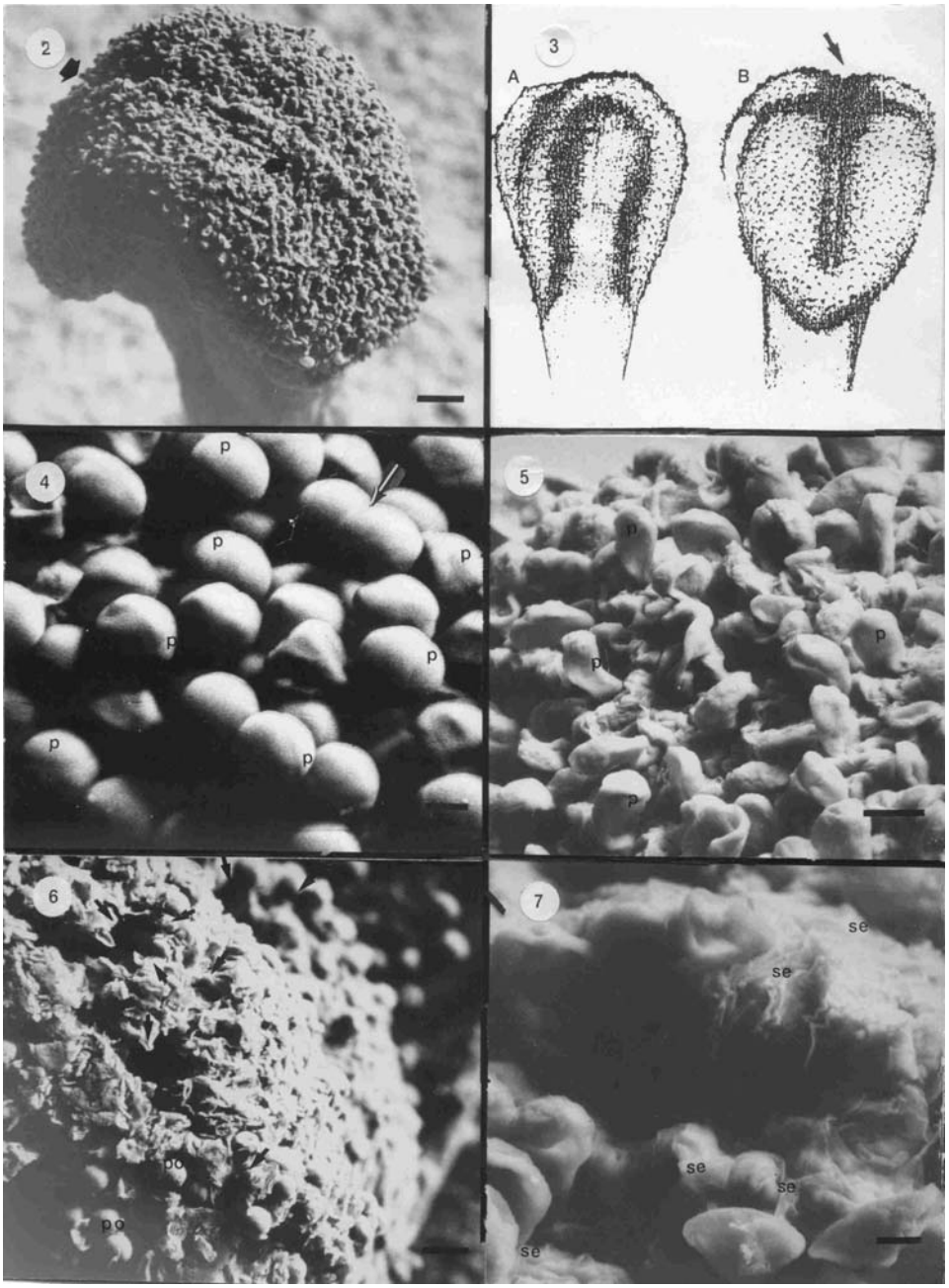
Early in floral development, after the floral apex flattened out as a result of growth occurring at the periphery, sepals were first to arise and grow rapidly in the buds of less than 1 mm in length (Fig. 8). Then petal, stamen and carpel initiation began (Fig. 9). The petal and stamen primodia appear at about the same time. Carpels initiated later as two separate entities and grew rapidly (Fig. 10). As the two carpels became closer in the buds of 2-3 mm, periclinal divisions occurred in the epidermal cells at the internal faces of the carpels and then extended to the tips (Fig. 11). Each epidermal cell may divide many times periclinally as well as anticlinally. The cells resulting from epidermal divisions also divided periclinally and anticlinally. However, there was a greater tendency to periclinal divisions (Fig. 12).

The continuous mitotic divisions of the epidermal cells and their derivatives gradually caused formation of ancestors of two new tissues in 5-6 mm long buds (Fig. 13); 1) Transmitting tissue in the center of the two carpels; 2) Stigmatic tissue at the tip of carpels. Subsequently in 9-10 mm long buds the developing carpels became elongated at the upper portion of the ovary, fused together and formed a short style terminated by stigmatic region (Fig. 14). At this stage transmitting tissue and stigma were clearly evident. The transmitting tissue consisted of narrow cells and originated from the epidermis. The stigma was composed of one layer of vacuolated epidermal cells transformed into papillae and 3-5 layers of small cells that were arranged tangenitally beneath them. The stigmatic tissue as well as transmitting tissue was epidermal in origin. The further development and maturation of the stigma continued until pollination.

The mature stigma consisted of two distinct zones; 1) Superficial zone; formed of papillae and 6-8 layers of secretory cells immediately beneath them. This zone was subjected to a gradual lysis during the ontogeny of the stigma. 2) Internal zone; formed of transmitting tissue of the style. This zone remains intact throughout the development. It also showed secretory activity (Fig. 15).

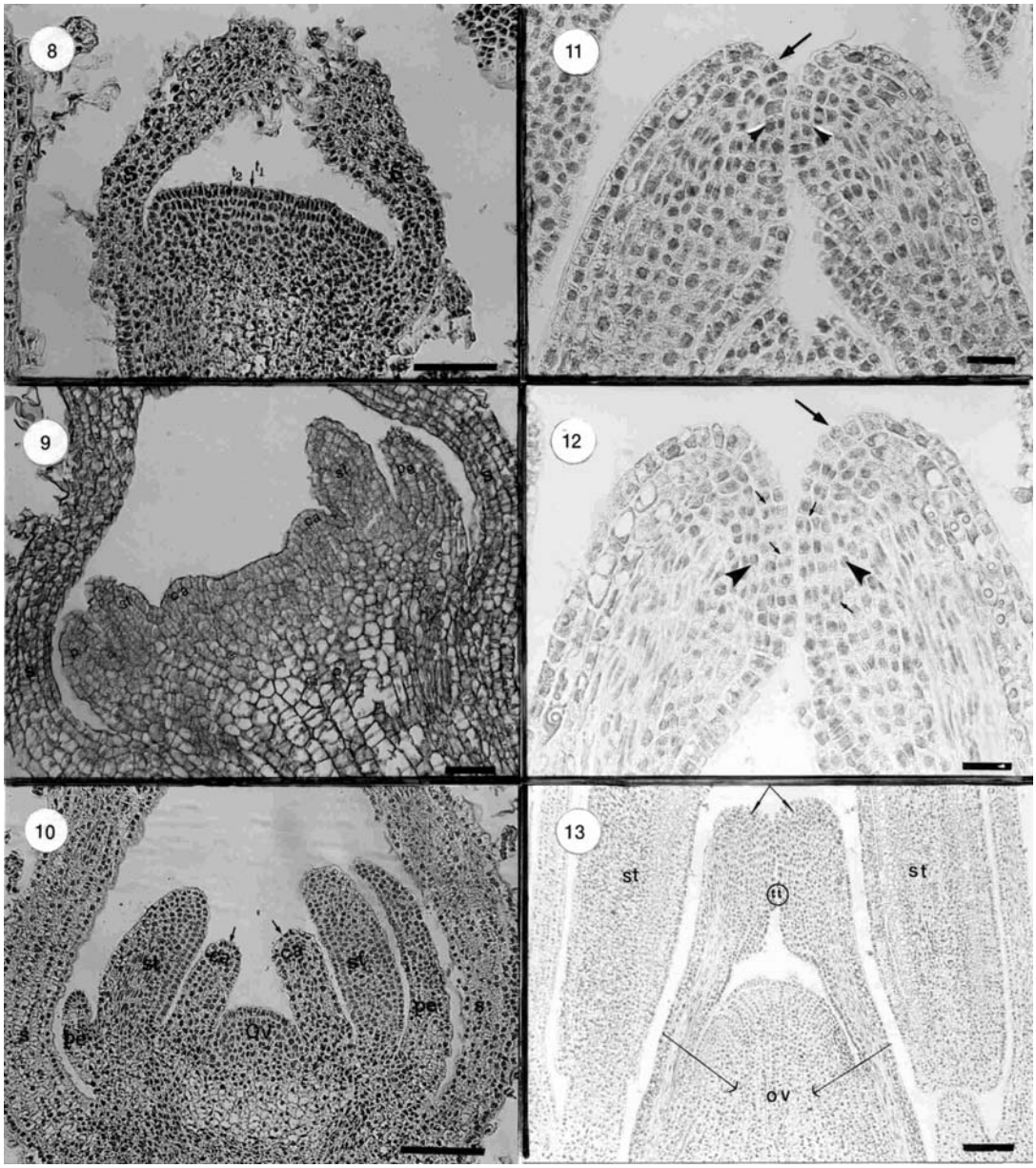
### Papillae Cells

Papillae cells, differentiated very early in the ontogeny of the stigma. Epidermal cells at the apex of young style (8-9 mm buds) expanded and vacuolized remarkably (Fig. 16). These epidermal cells enlarged and separated from one another and transformed into individual papillae in 9-10 mm long buds (see Fig. 14). In the course of development, they become elongated and enlarged considerably at the base. Then, they lysed and detached from the stigma surface. Detachment of papillae was related to the secretory activity of the stigma. Secretory products filled the intercellular spaces and covered the base of the papillae (Fig. 17). Thus, the junction between the cells that was mediated by cell walls was disrupted toward pollination. This process caused loose arrangement and gradual detachment of papillae and so their number decreased on the mature and receptive stigma.




**Figures 2-7.** Surface views of developing and mature stigma. 2. Overview of a mature stigma of *Datura stramonium* in SEM. Arrow indicates central depression. ×200. Bar=50 µm. 3. Diagrammatic aspect of the stigma of *Datura stramonium* in dorsal (A), and ventral (B) faces. Arrow indicates central depression. 4. Scanning electron micrograph of young papillae in the early stage of development (15-20 mm long buds). Papillae were bulliform in shape and densely arranged. Arrow indicates an anticlinally dividing papillae. ×1000. Bar=10 µm. 5. Scanning electron micrograph of a mature stigma. Note the thumb shaped papillae showing heterogeneous aspect because of the secretory activity and lysis phenomena. ×1300. Bar=10 µm. 6. Scanning electron micrograph of stigma; indicating surface pits and grooves (arrows), marking point of lysis and detachment of papillae and underlying secretory cells in pollinated stigma of the opened flowers. ×400. Bar=25 µm. 7. Scanning electron micrograph indicating free secretion particularly in interstices between papillae cells (arrows) embedding the base of the cells. ×2000. Bar=5 µm.

Abbreviations: p, papillae; se, secretion; po, pollen.




**Figures 8-13.** Sectional views of developing stigma and transmitting tissue. 8. Median Longitudinal section through apex in the bud of less than 1 mm in length. Note the flattened apex with biseriate tunica (t1, t2). Sepals showed a fast growth. There was no indication of other floral organ initiation. ×160. Bar=100 µm. 9. Median longitudinal section through apex in the buds of 1-2 mm in length, indicating floral organs initiation and development. ×250. Bar=40 µm. 10. Median longitudinal section through apex in the bud of 1-2 mm length. Pistil was constituted of two carpels. Arrows indicate terminal portion of the carpels which will elongate and widen considerably giving rise to style and stigma in the next stages of the development. ×165. Bar=100 µm. 11. Median longitudinal section through pistil in the buds of 2-3 mm length. Stigma originate after periclinal divisions of epidermis at the tip of the carpels (arrow). Note biseriate epidermis in ventral faces of the carpels (arrow heads) which is in the origin of transmitting tissue. ×400. Bar=25 µm. 12. Median longitudinal section through pistil in 4-5 mm long bud. Thickening of epidermis after periclinal division (small arrows) was remarkable in the ventral faces of the carpels (arrow heads). Epidermis was biseriate near the apex (arrow heads). ×400. Bar=25 µm. 13. Median longitudinal section through pistil in 5-6 mm long bud. The small apical cells (arrow) were the ancestors of the stigma. The centrally located cells in the style were first transmitting tissue cells. ×100. Bar=100 µm.

Abbreviations: ca, carpel; ov, ovary; pa, parenchyma; pe, petal; s, sepal; st, stamen; tt, transmitting tissue.

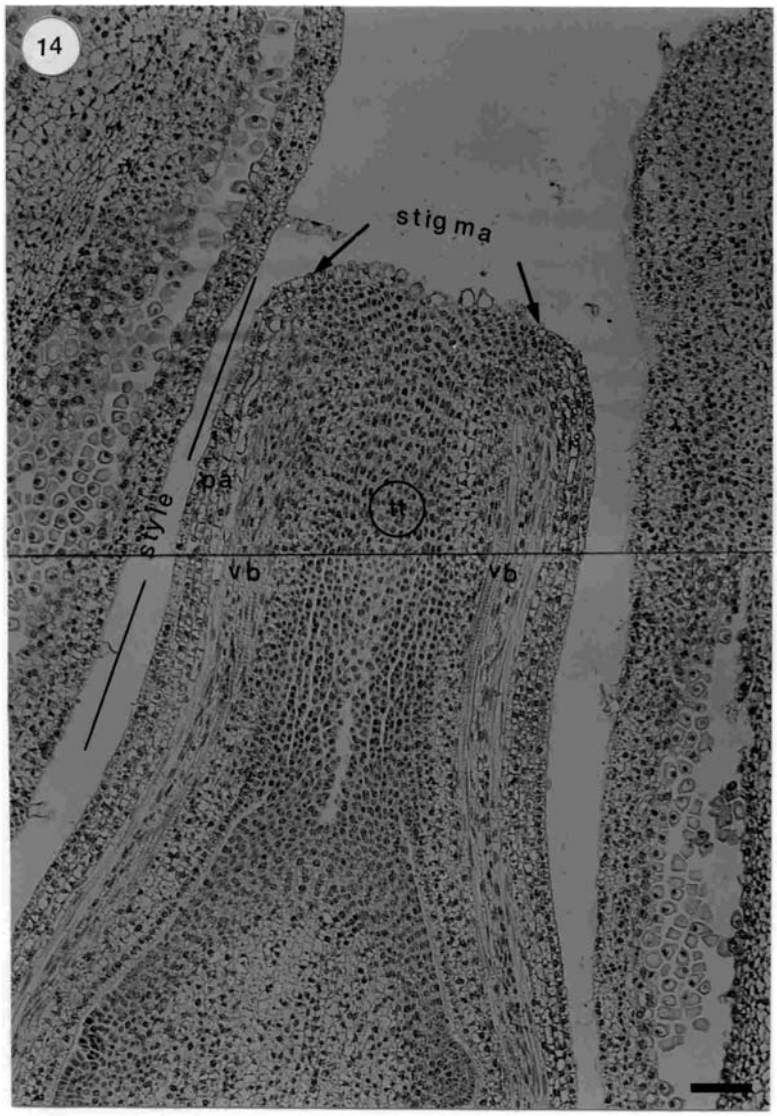


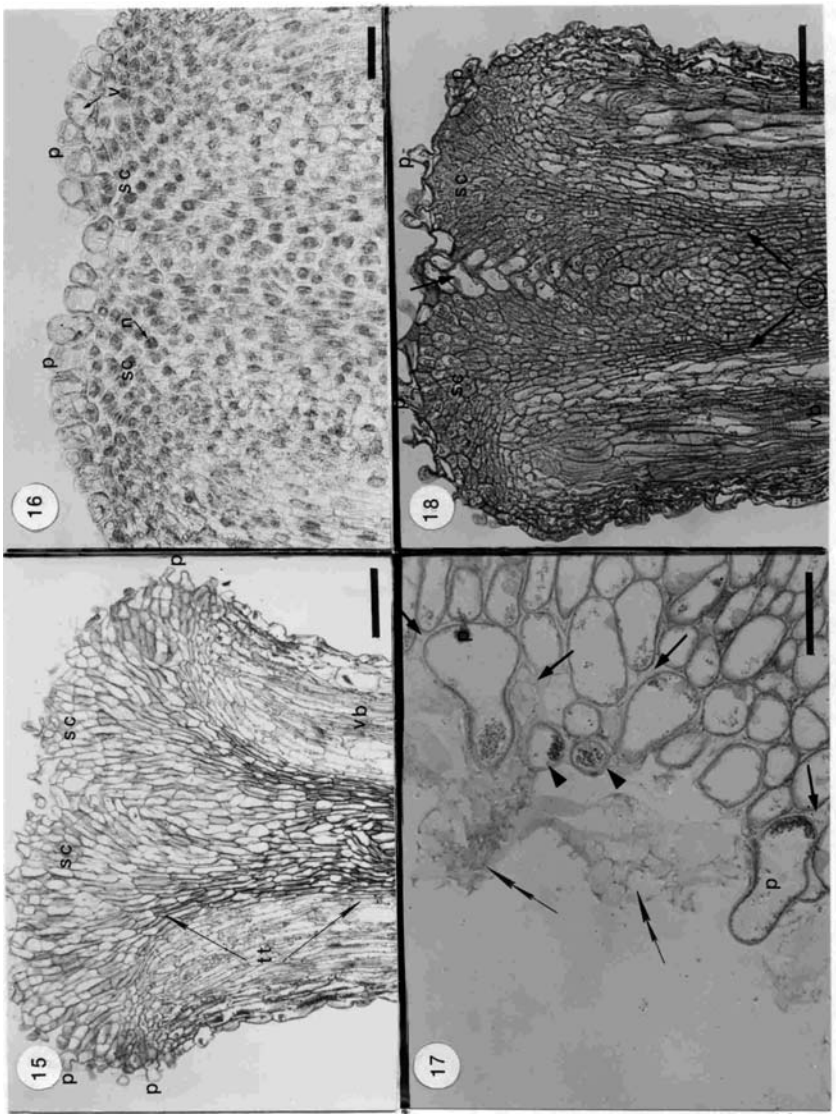

**Figure 14.** Median longitudinal section through the pistil in 9-10 mm long bud, showing the short style and the young papillae stigma. The style had a cortical parenchyma that envelop the transmitting tissue. Two vascular bundles transverse the parenchyma and ended beneath the stigma. ×250. Bar=40 µm.

Abbreviations: pa, parenchyma; tt, transmitting tissue; vb, vascular bundles.

**Secretory Cells**

The time span of the differentiation of secretory cells in the central *vs.* the peripheral part of the stigma was different. The secretory cells at the tip and central region of the stigma were the first to differentiate, appearing first in the buds of 15-20 mm long. Differentiation began with fast vacuolization and remarkable increase in cell size (Fig. 18). These cells from structural specifications point of view, especially rapid differentiation, are comparable with papillae. Peripherally located secretory cells started to differentiate late in the ontogeny of the stigma and they usually kept their small size in buds up to 25 mm long. At this stage, they became larger and vacuolized (Fig. 19). Thus, each differentiated secretory cell contained one large central vacuole, reduced but dense cytoplasm, and one large nucleus deeply stained with feulgen reaction (Fig. 20).

Time was the important factor in the developmental changes of the centrally located and other stigmatic cells




**Figures 15-18.** Sectional views of stigma and transmitting tissue in different stages of development. 15. Median longitudinal section (perpendicular to central depression) through the stigma in 40-45 mm long buds, in indicating its structural characteristics in maturity. ×65. Bar=200 µm. 16. Median longitudinal section from the tip of a young style in 8-9 mm long bud, showing early stage of papillae differentiation characterized by cell enlargement and vacuolization. The small cells with large and intensely stained nucleus beneath the papillae were the non differentiated secretory cells of the stigma. ×400. Bar=25 µm. 17. Thin section from papillae and secretory cells of a mature stigma. A large amount of secretion (arrows) was present between papillae and secretory cells. Secretion had a bubbly appearance on the stigma (double arrows). Note the section of free cells in the stigmatic secretion (arrow heads). ×320. Bar=50 µm. 18. Median longitudinal section (perpendicular to central depression) through the stigma in 15-20 mm long buds. In the early stage of stigma development two distinct regions became visible: A central region (arrow) consisting of large vacuolized secretory cells and a surrounding peripheral region consisting of undifferentiated small cells and developed papillae. Note two vascular bundles traversing the cortical parenchyma cells of the style and terminating at the base of the stigma. ×320. Bar=50 µm.

Abbreviations: n, nucleus; p, papillae; sc, secretory cells; tt, transmitting tissue; v, vacuole; vb, vascular bundle.

at the level of light microscopy. There were occasionally large cells with smaller vacuoles and more cytoplasm among vacuolated cells of the stigma (Fig. 20). Other important features of differentiation of the secretory cells were in regard to the changes that occurred in their arrangement and attachment to each other. In the course of development, the secretory cells became elongated and arranged perpendicularly to the stigma surface. Those located in the central region were irregularly interlocked (Fig. 15). Furthermore, the secretory activity of the stigmatic cells and accumulation of this product in intercellular regions caused closely attached secretory cells in the first stages of the development gradually became distant from each other. This process happened especially in the level of anticlinal walls(Figs. 15, 20).

Secretory cells, as well as papillae, underwent lysis during ontogeny of the stigma. The process began at first in central cells of the stigma in ≅ 20 mm long buds, then extended basipetally in this region. Thus the stigma in opened flowers became divided into two separate lobes. Lysis and cell detachment occurred later in the other cells of the stigma. The most obvious features of cells destined to lysis were enlargement followed by the cell wall dissolution. There was no remarkable organelles degradation at the level of light microscopy. Pollination occurred well before anthesis but the lysis process continued up to anthesis and the senescence of the stigma. This phenomenon became more pronounced after pollen grain germination and pollen tube growth in opened flowers (Figs. 21, 22). In the late stages of development, the stigma lost its rigidity and became a degraded lacunar, gel-like tissue that was easily removed by a spatula. This loosen tissue was supported by the compact transmitting tissue of the style.

**Transmitting Tissue**

Transmitting tissue of the style branch equally beneath the stigma into two, with each branch expanded out into the stigma. This tissue came into direct contact with secretory cells and constitute an integrated part of the stigma structure (Figs. 15, 18). It is compact tissue, formed by narrow and elongated cells with tapering ends. These cells had thick lateral walls and stained deeply for carbohydrates (PAS reaction) (Figs. 15, 18). Transmitting tissue showed the aspect of collenchyma in cross sections (Fig. 21).

## Discussion

Stigma and transmitting tissues of *Datura stramonium* were found to be strictly epidermal in origin, as reported earlier by Satina [15]. Four stages were identified in the present study in *Datura*'s stigma life cycle arbitrarily based on the length of the floral buds:

1) Histogenesis; comprised of buds of 2-15 mm in length. In this stage, the stigmatic cells were meristemic and mitotic divisions continued in up to 15 mm long buds.

2) Growth and development; comprised of buds of 15-40 mm in length. In this stage, cell divisions were mostly ended and differentiation began. Differentiation was characterized by an increase in cell size and important histological and histochemical changes.

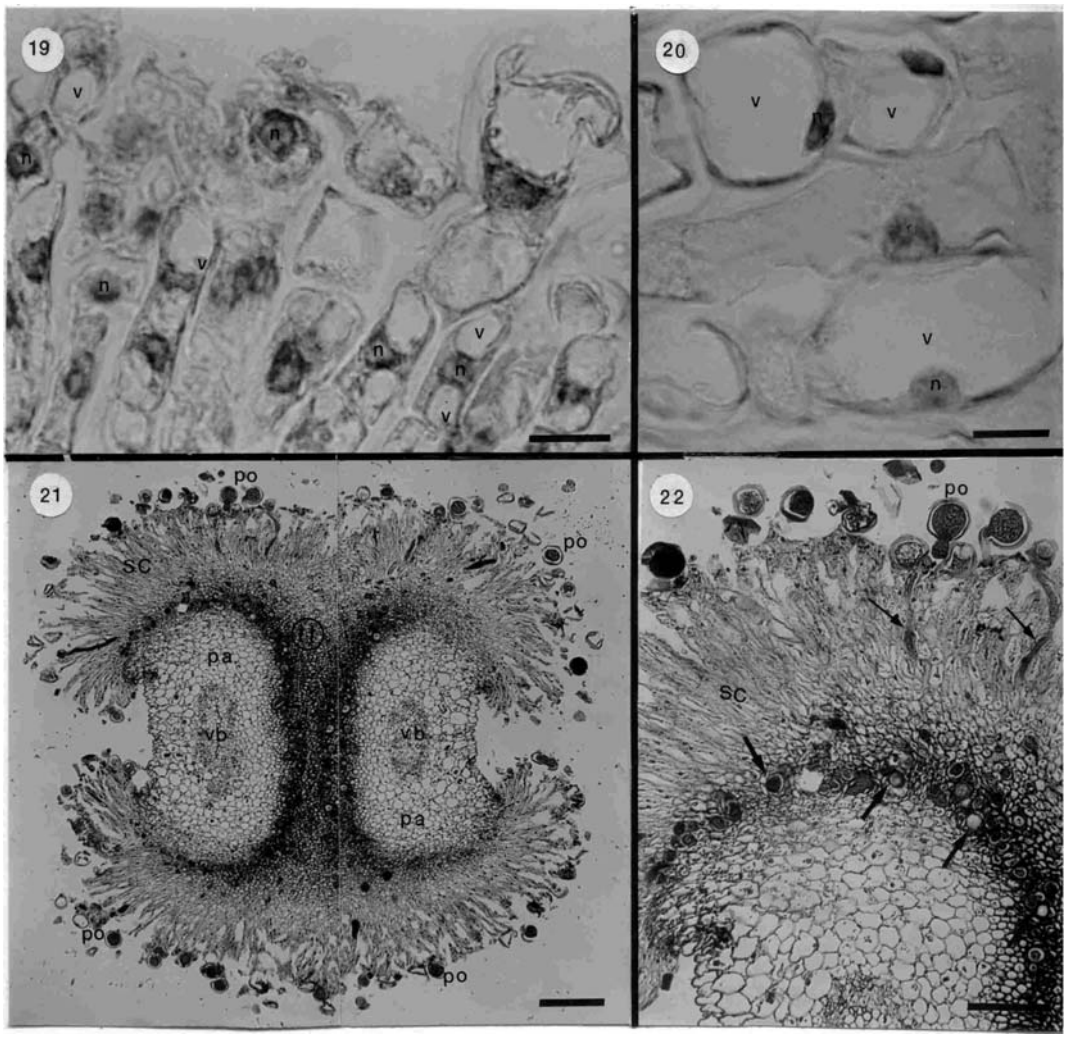
3) Maturity; comprised of buds of 40-60 mm in length. During this stage the stigma anatomically and histochemically became pollen receptive. Stigma receptivity was before anthesis.

4) Post pollination and senescence; comprised of buds more than 40-60 mm – dependent on the environmental conditions – to the opened flowers. The environmental conditions such as cold weather, dryness, and short day cause rapid pollination. During this stage, the stigma gradually lost its integrity and disrupted.

The wet type stigma of *Datura* was formed as a result of stigma secretion, lysis and detachment of the papillae and secretory cells. The light microscopy and particularly scanning electron microscopy revealed that the superficial cells of *Datura*'s stigma were not, in fact, attached. These cells eventually developed into papillae (Fig. 5). The papillae started to lysis and fall off at second and third stages of development. At the fourth stage, most of the papillae as well as upper superficial secretory cells were detached. This caused the stigma surface to look spongy. It was of interest that at the fourth stage, there were still some intact papillae. These papillae were mostly in the basal parts of the stigma of the opened flowers. These observations suggested that papillae on the stigma surface have a limited life cycle; ones that differentiated early, become lysed and detached soon (at stages 2 and 3 of the ontogeny) and the fewer papillae that differentiated late, remained intact until the last stages in the ontogeny of the stigma (stage 4). In our opinion, increasing amount of stigma secretion and its ascending flow was an important factor for the detachment of the papillae and secretory cells of the stigma.

Anatomical structure of the stigma of *Datura stramonium* was in accordance to the Dumas's concept [2], on wet stigma structure. It consisted of distinct zones (described previously). The present study revealed that the organization of stigma in *Datura* was very similar to that of *Lycopersicon peruvianum* [3] and especially to commercial potato, *Solanum tuberosuml*, cv shepody [11].

A remarkable characteristic of the secretory cells was their loose arrangement in the mature state, and cell lysis which occurs particularly after pollination. The papillae as well as the superficial secretory cells in *Datura stramonium* were subjected to lysis and




**Figures 19-22.** Maturation of stigma in sectional view. 19. Portion of secretory cells in 25-30 mm long buds indicating the beginning of vacuolization. ×1600. Bar=10 µm. 20. Portion of secretory cells in 60-65 mm buds indicating considerable vacuolization, increase in size and large nucleus. It was notable that some secretory cells may reserve their dense cytoplasm for a long time. ×1600. Bar=10 µm. 21. Transverse section of stigma indicating the secretory cells separated and lysed in the pollinated, opened flowers. It is a loosen tissue in this stage. ×65. Bar=200 µm. 22. Higher magnification of a portion of the stigma from Fig. 21, note pollen tubes growing between stigmatic cells (small arrows) and transmitting tissue cells (large arrows). ×160. Bar=100 µm.

Abbreviations: n, nucleus; pa, parenchyma; po, pollen; sc, secretory cells; tt, transmitting tissue; v, vacuole; vb, vascular bundle.

detachment. Papillae degeneration was observed in many other species at anthesis [11], and at the time of full expansion of flowers, after natural pollination by insect [16]. In *Datura stramonium* papillae lysis and cell detachment occurred in the young stigma (stage 2) and continued up to maturity and pollination (stages 3 and 4). There were no signs of organelles degeneration in the papillae or in the secretory cells. Cell wall dissolution and cell detachment – occurred with increasing of secretory products – seemed to be the major causes of stigma disruption.

*Datura*'s stigma was classified as wet, nonpapillae one by Heslop-Harrison and Shivanna [5] (Group IV). But, they did not specify the species of *Datura*. The present study suggests that the stigma of *Datura stramonium* can be considered as a wet papillae type (their Group III). Since the papillae detachment occurred by the time the flowers opened, the papillae nature of the stigma may have been previously overlooked.

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