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LIPIDS IN THE STIGMATIC SECRETION OF DATUR STRAMONIUM L (SOLANACEAE)*

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Abstract – The chemical nature of secretion by the stigma and transmitting tissue in Datura stramonium was studied by light microscopy using Sudan black B, Coomasie brilliant blue (CBB), Toluidine blue, Safranin, and PAS. The stigmatic secretion reacted positively for lipids. During development, stigmatic cells became separated and intracellular lipids were transferred to intercellular spaces where they gradually migrated to the stigma surface. Excretion of the lipids was a continuous process and persisted during maturity and even in pollinated and opened flowers (post-pollinated). Lipid secretion was associated with cell autolysis in papillae and in secretory cells of the stigma, particularly in superficially located ones. The transmitting tissue cells also showed glandular activity with the release of a polysaccharide rich substance. The pollen germination and pollen tube growth seem to be closely dependent upon these secretions in Datura stramonium. The different nature of the secretion of the stigma (lipids) versus secretion of the transmitting tissue of the style (carbohydrates), supports the hypothesis that the pollen tube metabolism changes from autotrophic to the heterotrophic upon entering the transmitting tissue from the stigma surface.

Keywords – Datura stramonium, secretory cells, lipids, carbohydrates, pollination, stigma, transmitting tissue, papillae, compatibility

1. INTRODUCTION

The morphology and anatomy of the stigma in Datura stramonium was described using light and scanning electron microscopy [1]. The stigma in this species was classified as wet, papillated (Group III), and covered with sticky secretion. The extracellular matrix of stigma and stylar transmitting tissue, enriched with secretory materials, is the path for the pollen tube. It provides the chemical and physical support as well as directional cues for pollen tube extension toward the ovules [2-5]. Although pollen has a high capacity to support its own activity during germination and tube growth [6, 7], the pistil also contributes to this process. The differences between in vivo and in vitro pollen tube growth indicate a major contribution of chemical and/or physical factors from pistil to pollen tube growth in vivo [8-10].

Recent studies on different aspects of reproduction in flowering plants have highlighted the importance of pistil in sexual reproduction. In studies and investigations carried out on ultrastructure, cytochemistry [11-13], immunocytochemistry and the details of pollen-pistil interaction [14-19], the transmitting tissue is the central subject.

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The focus of the present study was on secretory activity of the stigma and transmitting tissue. The stigmatic secretion of flowering plants is composed of a large number of substances.The composition varies among species, but consists mainly of lipids and phenolic compounds [20]. Previous studies have suggested that the lipidic secretion of the stigma of many species has no role in the nutrition of the pollen tube, while the polysaccharidic intercellular substance of the transmitting tissue plays a major function in this regard [21- 24]. The objective of this study was to use light microscopy and histochemistry to determine whether in a self-pollinating plant, Datura, there are differences in the secretion process and products of the stigma versus the transmitting tissue

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2. MATERIALS AND METHODS

Datura stramonium, which flowers in four different stages of floral development- defined in the previous work as histogenesis; growth and development; maturation; pollination and senescence [1], were obtained for fixation. A minimum of 100 flowers were collected from wild populations in the North of Tabriz, Iran.

Cytochemical Techniques for Light Microscopy

For lipid detection the stigmas were fixed in Meve fixative for one hour at room temperature (RT) [25], dehydrated through an ethanol series, and embedded in paraffin. Sections were then cut at 5-12 μm and stained with Sudan black B. Fresh sections were also cut and stained with Sudan black B and Sudan III [26].

For localizing total polysaccharides, the stigmas were fixed in 10% neutralized formaldehyde and dehydrated as previously mentioned. Sections were cut at 5-7 μm and stained with Periodic Acid Schiff (PAS) [26]. For lipid intra-assay control, fresh tissue sections of the stigma were treated with different lipid solvents. In addition, for the intra-assay control of polysaccharides, the acid hydrolysis stage in the PAS method was eliminated [26].

For other cytochemical studies, mature stigmas were fixed in formaldehyde calcium for 18 hours at RT, dehydrated in an ethanol series and embedded in paraffin. Sections were cut at 5-10 μm and were used to localize proteins and polyphenols.The presence of total proteins was detected by staining the sections with 0.02% Coomasie Brilliant Blue (CBB) in Clark solution (PH=2) [26].For identification of phenolic compounds, sections were stained: 1) with 0.02 % Toluidine blue for 15-30 s at 60 ^{0} C (modified from Feder and O'Brien) [27]; 2) A combination of Safranin in 50 % ethanol [28]. For each assay control fresh materials were also included. Slides were examined using a bright filed Zeiss photo microscopy.

3. RESULTS

The secretion products in wet papillate stigma of Datura stramonium were highly lipidic in nature. Stigmatic secretion reacted positively to stains used to test the presence of lipids in fixed and fresh materials. Histochemical studies on the stigma of Datura stramonium in four developmental stages revealed that the first stage (histogenesis, 2-15 mm long buds) was, in fact, a presecretory phase. In this stage stigma cells were essentially meristematic. At stage two (growth and development, 15-40 mm long buds), secretory activity began. The principal structural feature of this stage was the appearance of a great number of black particles in stigmatic cells after staining with Sudan black B. In addition, the lipidic nature of the stigma secretion was confirmed by staining with Sudan III on the fresh, hand cut materials, which revealed red-staining particles. Secretory activity started in 15-20

mm long buds. In this stage, papillae, secretory cells and transmitting tissue cells (with less importance) became filled with black particles which have been identified as lipids (Fig. 1). Excretion began fairly early in papillar cells. The small droplets of lipids fused together and accumulated beneath the plasmalemma (Fig. 2). Furthermore, the lipid secretion was observed in the cell wall just below the cuticle. There was also some papillae which lysed while still keeping their intracellular lipidic components (Fig. 3).

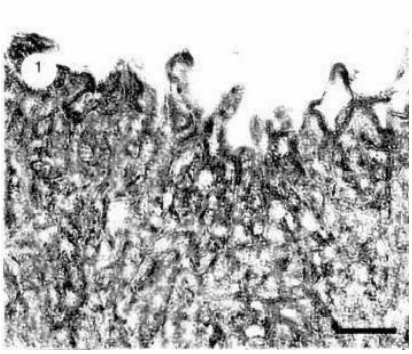

Fig. 1. Young stigma showing papillae and secretory cells
with small granules (arrows). X620.Bar=25 µm

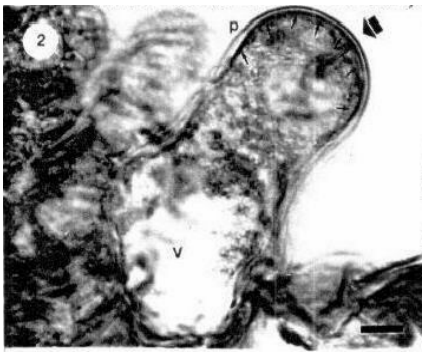

Fig. 2. Intensely colored cuticle and lipidic accumulation beneath it in a developing stigma.
The lipidic secretion was also visible in the form of a dark layer
beneath the plasmalemma (arrows). X1600. Bar=4 µm

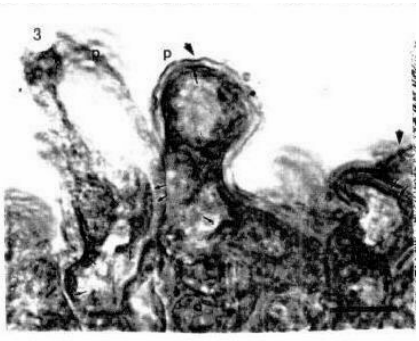

Fig. 3. Intracellular lipids (small arrows) and early formation of lipidic excretion beneath the ruptured
cuticle (arrow heads) in a papilla of a developing stigma. Note a lysing papilla while
Keeping intracellar lipids (small arrows). X1600.Bar=10 µm

There was no apparent export of lipid droplets up to the 20 mm long bud stage in secretory cells beneath the papillae. Lipid secretion appeared for the first time in buds more than 20 mm long in the

form of small droplets between the secretory cells of the stigma. Gradually, droplets increased in number and fused into larger masses of intercellular lipids (Fig. 4). This phenomenon was associated with progressive detachment of papillae and superficially located secretory cells, and with a marked decrease in lipid content of the secretory cells. In the mature stigma (buds of 40-60 mm long) the cells became mostly devoid of intracellular lipids. The lipid secretion then accumulated largely in intercellular spaces of anticlinal walls and migrated to the stigma surface (Fig. 5).The migration process continued until pollination. However at anthesis the lipidic secretion was observed in small amounts in the intercellular spaces.

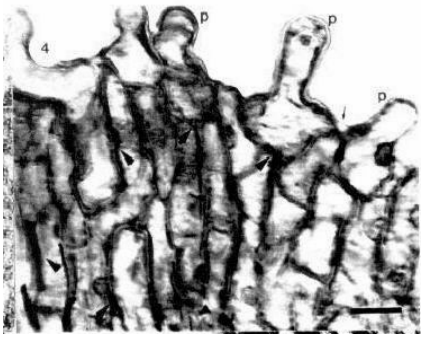

Fig. 4. Secretory cells in a developing stigma; indicating a small amount of secretion in the intracellular space (arrow heads). Note the disruption of the cuticle between the papillae (small arrows). X650. Bar=20 μm

Fig. 5. Secretory tissue in a mature stigma; indicating large amount of lipidic secretion in intercellular spaces (arrows). X500. Bar=20 μm

The onset of lipid production in the transmitting tissue was the same as in the stigmatic cells, but with only small amounts being synthesized. Furthermore, at the same time, the lipid particles were revealed in very limited numbers in the cortical parenchyma, vascular tissue and epidermal cells of the style. In the later stages, however, lipid secretion was never observed in the intercellular spaces of the above mentioned tissues. In a mature stigma, Sudan black B staining revealed two distinguishable regions; 1) Superficial zone: formed by secretory cells which were intensely stained with Sudan black B. 2) Internal zone: formed by transmitting tissue cells which did not stain at all for lipids (Fig. 6).

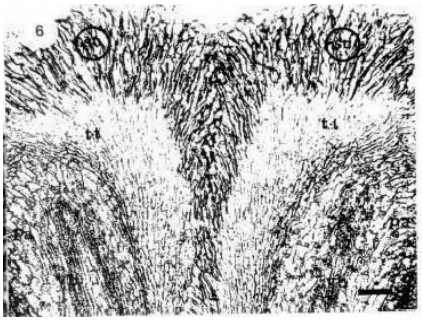

Fig. 6. Mature stigma showing two distinct regions with different secretory characteristics. X80. Bar=100 μm

The PAS staining for the detection of polysaccharides in the stigmatic secretion was weak, while the secretory cell walls showed strong staining (Fig. 7). Whereas thickened walls of the transmitting tissue cells and homogenous intercellular substance stained strongly with PAS (dark purple) and revealed the polysaccharidic nature of intercellular substance in this tissue (Fig. 8).

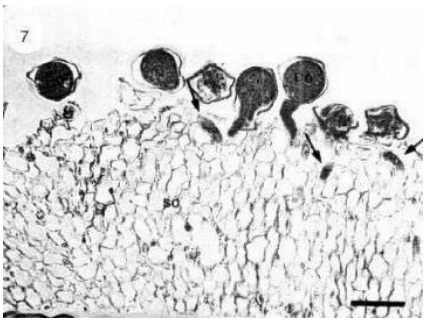

Fig. 7. Pollinated stigma showing intercellular substance reacted weakly. Note deeply stained pollen grains and pollen tubes (arrows). X250. Bar=50 μm

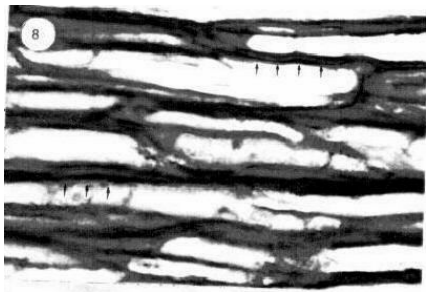

Fig. 8. Longitudinal section of transmitting tissue in 40-50 mm long buds; indicating thickened walls of this tissue deeply stained (arrows). X1600. Bar=10 μm

The secretory cells and transmitting tissue cells were devoid of starch grains in all stages of the stigma development (Figs. 7 & 8). However, there were great numbers of starch clusters in cortical parenchyma cells of the style (Fig. 9). There were other apparent storage bodies in the cells that did not react with starch and lipid staining reagents. Using PAS, numerous starch grains appeared as intensely stained granules in early developmental stages, particularly in dorsal parenchyma of the style. The starch content increased until the 35 mm bud stage, however the 4-5 layers of small parenchyma cells beneath the stigma always lacked starch grains (Fig. 10). Starch content markedly decreased in parenchyma cells so that upon reaching maturity, there was no starch in the style. This

phenomenon occurred first beneath the stigma, and then continued throughout the style basipetally. There was a remarkable correlation between the decrease in starch reserve in parenchyma cells and the intense staining of the transmitting tissue cell walls. The intercellular substance of transmitting tissue in the buds of 40-60 mm in length (mature stage) showed a heavy staining with PAS, unlike buds in earlier stages.

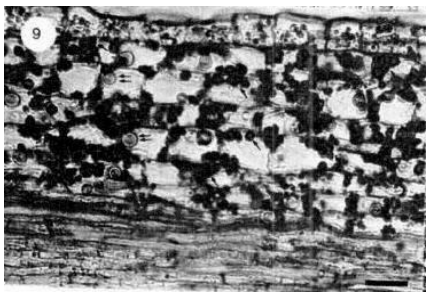

Fig. 9. Longitudinal section in the style of a 20-25 mm long bud. Parenchyma cells were rich in starch reserves (single arrows). Note accumulation of starch in dorsal parenchyma. Other possible storage bodies (double arrows) were visible. X200.Bar=50 μm

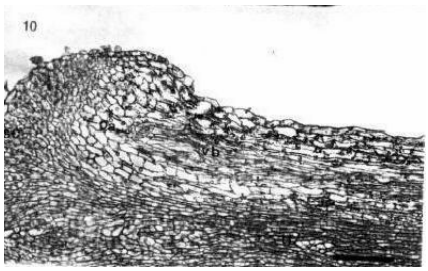

Fig. 10. Median longitudinal section in a 25-30 mm long bud; indicating the end part of the style and a portion of the stigma. Note the absence of starch reserves in the parenchyma cells below the stigma. X160. Bar=100 μm

No protein was localized in the stigmatic secretion after CBB staining. However, there were a great number of globular storage bodies in the secretory cells, as well as in the papillae that stained intensely with CBB (Fig. 11). These storage bodies were unlikely to be lipoproteins or glycoproteins as they showed no reaction with lipid or polysaccharide reagents.

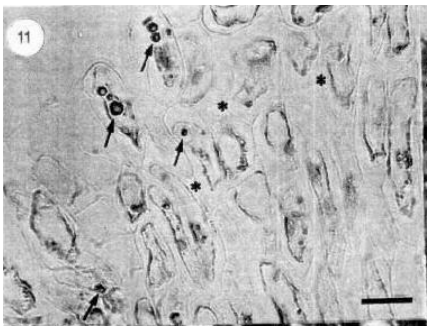

Fig. 11. Secretory cells of a mature stigma stained with Coomasie brilliant blue; indicating the absence of staining in intercellular substance (asterisks). Note the intravacuolar globules stained positively (arrows). X625. Bar=20 μm

Different histochemical procedures for identification of different phenolic compounds in stigmatic secretion did not result in a positive reaction. But the globular storage bodies stained

positively with Toluidine Blue (Fig. 12) and Safranin (Fig. 13 & 14). The staining characteristics of these storage bodies suggested their complex proteopolyphenolic nature.

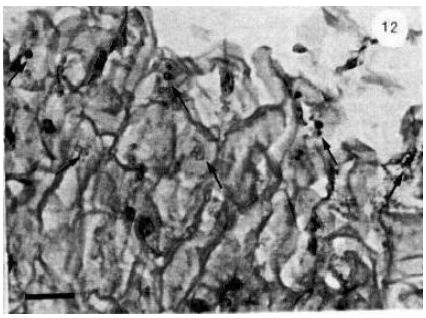

Fig. 12. Secretory cells of a mature stigma stained with Toluidine blue; indicating the absence of staining in intercellular substance for polyphenols. Note the intravacuolar globules stained positively (arrows). X625. Bar=20 µm

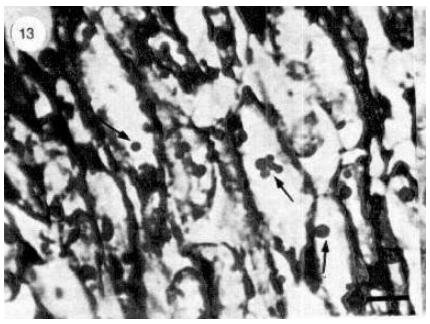

Fig. 13. Secretory cells of mature stigma stained with Safranin; revealing polyphenols. Note the intravacuolar globules stained positively (arrows). X1000. Bar=10 µm

Fig. 14. Secretory cells of mature stigma stained with Safranin; Note the intensely stained globules (arrows) released out of the cells after autolysis. X1600. Bar=10 µm

4. DISCUSSION

The present study further confirms the wet, papillate nature of the stigma of Datura stramonium, first described by Aliasgharpour et al [1]. The histochemical investigation with Sudan black B, as well as with Sudan III, indicated the essentially lipidic nature of the stigma secretion.This study also showed the secretory activity of Datura's stigma, as well as its anatomical features, can be studied in the four stages previously discussed [1]. In this regard, the first histogenesis stage (2-15 mm long buds) was considered as the lipid presynthetic phase. Lipids were synthesized at the second stage, growth and development (15-40 mm long buds). At the beginning of this stage the lipids were synthesized in

considerable amounts in the stigmatic cells, and then secreted out of the cells, accumulated between them and migrated gradually to the stigma surface. During the third stage, maturity, (40-60 mm long buds) lipid secretions continued to migrate to the stigma surface. In this stage there was a remarkable amount of exudates on the stigma. At the fourth stage, (pollination and senescence) there was no further secretory activity. Nevertheless, the small amount of lipid secretion that remained in the intercellular spaces revealed the gradual and continuous nature of secretion in Datura tramonium.

Many species of the Solanaceae and other families were reported to contain lipids in their stigmatic secretion [29-37]. This study revealed that besides the anatomical resemblance between the stigma of Datura and Solanum tuberosum [1], the stigmatic secretion of Datura was essentially lipidic in nature, as was observed in Solanum tuberosum [29]. The lipids of the stigma of Datura may have a complex structure. Using different control methods for determination of their consistency, lipids did not dissolve completely and the control sections were always stained; except for the samples that were incubated for at least 5 days in a mixture of chloroform- methanol (2:1 v/v) solvent.

The lipidic secretion of the stigma is reported to act not only as a liquid cuticle, controlling excessive transpiration [31, 32], but also as a protective layer against different environmental changes [29]. In addition, it was suggested that the lipids take part in trapping pollen grains because of the particular physical properties, but they do not have any role in supplying nutrition to the pollen [32]. The presence of a fluid on the stigmatic surface of flowers is reported to influence pollen germination [38].

Datura stramonium is a self compatible species (>95%) with variation in outcrossing rate, which is correlated in particular with floral morphology [39, 40].Considering the fact that pollination in this species occurs in the bud stage, the lipid secretion may not be so important in protection against environmental factors such as washing off by rain, desiccation and/or other factors. Sanders and Lord [41] indicated that in compatible pollination adhesion between the pollen tube and stigma and/or stylar transmitting tissue is an essential part of this interaction. The adhesion event was considered partly responsible for the fast and guided progress of the tube cells to the ovary [42]. More recent studies on the stigma of several species indicated the implication of certain lipids in directional pollon tube growth [43]. It is not unreasonable to assume that the lipids of the stigma in Datura would, in a way, serve as an adhesive component which ensures the directional pollen tube growth on the stigma surface. We believe however, that the lipidic stigma secretion is more likely to be involved in wetting, as well as regulating water for the pollen grain necessary for its germination. This is in accordance with Vasil [44]. In a very recent study [45], the role of glycerol and probably other lipids as a matrix in which water molecules are distributed or that modifies the membrane permeability of the pollen at the site of tube formation is discussed. Our findings on the different nature of secretion in the stigmatic secretory cells and transmitting tissue cells were in accordance with Herrero and Dickinson [22] and Arbeloa and Herreero [46]. They mentioned a change from the autotrophic life of the pollen tube in the stigma- where it depends on its own stored nutrients- to the heterotrophic form in the style.

The PAS method revealed the absence of starch grains in the secretory cells as well as in the papillae and transmitting tissue cells. However, cortical parenchyma of the style showed a considerable accumulation of starch. Starch synthesis started from the beginning of the developmental stage (almost simultaneously with lipid synthesis) in the parenchyma cells. The number of starch grains increased up to 30-35 mm long buds, then decreased gradually after until maturity, where there were no starch grains left. It was interesting that concurrent with this phenomenon, the intercellular substance of transmitting tissue showed an increased staining with PAS. These observations suggested that the starch grains may be consumed by transmitting tissue cells near maturity, causing

the increase of the polysaccharidic content of an intercellular secretory substance at the mature stage. A similar situation has been previously reported by Clifford and Owens, [47] while studying the transmitting tissue in the Ocidiinae. The carbohydrates probably represent a nutrient source for the pollen tube as it grows through the intercellular spaces of the transmitting tissue. Furthermore, the trophic dependance of the pollen tube is important in regulating pollen tube kinetics [48] and in reducing the gametophitic population [49]. In addition, it is indicated that this interaction is likely involved in the selection of particular pollen genotypes by the pistil [50].

Histochemical studies for the detection of proteins and polyphenols revealed the presence of proteopolyphenolic reserves observed in the form of small globules in papillae and secretory cells. They were never excreted out of the cells in intercellular spaces. They remained in the vacuoles until the cell lysed. Cell autolysis was a common process in the stigma of Datura stramonium [1]. This phenomenon was associated with the liberation of intracellular content, as well as proteopolyphenolic globules in the stigmatic germination medium. To our knowledge, this is the only example of localization of intracellular protein binding polyphenols in the secretory product of the stigma. The significance of the presence of these complex compounds was not clear, however the proteic component of the stigma [20], secretion was known to have an important role in pollen-stigma interactions.

Flowers of Datura stramonium are self-compatible. Phenolic compounds were known to have a stimulatory or inhibitory effect on pollen germination [44]. The presence of polyphenols in the surface exudates of stigma may be regarded as a stimulatory factor. Furthermore, polyphenols may be toxic to bacteria [51, 52], fungi [53] and insects [54]. Considering the total autolysis of secretory cells of stigma in the fourth stage of its development, especially in opened flowers, the protective effect of polyphenols is also probable. The presence of polyphenols in the stigmatic surface exudate may be a part of an effective mechanism to protect against biotic stresses and to assure the performance of a successful reproduction in Datura stramonium, manifested by the formation of capsules containing from 100 to > 500 seeds.

To conclude, intercellular secretion of stigma in Datura srtamonium has, essentially, a lipidic nature. In fact, the germination of pollen and pollen tube growth takes place in a complex medium. This medium is a gel-like soft bed which pollen grains dip into and provides all the demanded physical conditions and chemical factors necessary for pollen germination and pollen tube growth. Further studies to elucidate the role of compounds of the stigmatic surface are in progress. We are currently collecting additional chemical and ultra-structural information in order to provide a firmly basic data to pave the way in future studies in pollen/pistil interaction in Datura stramonium.

NOMENCLATURE

p	papillae
pa	parenchyma
po	pollen
sc	secretory cells
tt	transmitting tissue
vb	vascular bundle

REFERENCES

1. Aliasgharpour, M., Hekmatshoar, H. & Hosseyni, M. S. (2000). Stigma of Datura stramonium L. (Solanaceae): istogenesis, morphology and developmental anatomy, *J. Sci. I. R. of Iran*, 11, 267.
2. Mascarenhas, J. P. (1979). The biochemistry of angiosperm pollen development*, Bot. Rev*. 41, 259.
3. Marcarenhas, J. P. (1993). Molecular mechanisms of pollen tube growth and differentiation, *Plant cell*, 5, 1303.
4. Heslop-Harrison, J. (1987). Pollen germination and pollen tube growth, *Int. Rev. Cytol*, 107, 1.
5. Lord, E. M. & Sanders, L. C. (1992). Roles for the extracellular matrix in plant development and pollination: a special case of cell movement in plants*, Dev Biol*, 153, 16.
6. Vasil, I. K. (1987). Physiology and culture of pollen, Int. *Rev. Cytol*., 107, 127.
7. Steer, M. W. & Steer, J. M. (1989). Pollen tube tip growth*, New. Phytol*., 111, 323 .
8. Heslop-Harrison, Y., Heslop-Harison, J. & Reger, B. (1985). Pollen tube guidance and the regulation of the number in Zea mays, Acta Bot. *Neerl.,* 34, 193.
9. Lush, W. M., Opat, A, Nie, F. & Clarke, A.E. (1997). An assay for assessing the effect of growth factors on N.alata pollen tube in culture, *Sex Plant Reprod*., 10, 351.
10. Cheung, A. Y. & Wu, H. M. (1999). Arabinogalactan proteins in plant sexual reproduction, *Protoplasma*., 208, 87.
11. Ciampolini, F., Saleri, C., Di Pietro, D. & Cresti, M. (1996). Structural and cytochemical characteristics of the stigma and style in vitis vinifera L. var. sangiovese (vitaceae), *Annals of Bot*., 78, 759.
12. Ciampolini F. & Cresti, M. (1998). The structure and cytochemistry of the stigma-style complex of corylus ovellana L "Tonda Gentile delle L anghe" (Corylaceae). *Annals of Bot*., 81, 513.
13. Lennon, K. A., Roy, S. J., Hepler, P. K. & Lord, E. M. (1998). The structure of the transmitting tissue of Arabidopsis Thaliana (L.) and the path of pollen tube growth, *Sex Plant Reprod*., 11, 49.
14. Bell, P. R. (1995). Incompatibility in flowering plants: adaptation of an ancient response, *Plant cell*, 7, p. 5.
15. Franklin, F. C., Lawrence, Franklin, J. J. & Tong, V. E. (1995). Cell and molecular biology of self-incompatibility in flowering plants. Int. *Rev. Cytol*, 158, 4.
16. De Nettancourt, D. (1997). Incompatibility in angiosperms, *Sex Plant Reprod*., 10, 158.
17. Gupta, Pramila, Shivanna, K. R. & Ran Mohan, H. Y. (1998).Pollen-Pistil interaction in non-pseudogamous Apomict, Commiphora Wightii. *Annals of Bot*., 81, 589.
18. Mathews, M. L., Gardner, J. & Sedgley, M. (1999). The relationship between transmitting tissue, pollen tube, and ovule number: A study across 10 angiosperm families, *Int. J. Plant Sci*., 160, 673.
19. Mallet, J. C., Park, S. Y., Nothnagel, E. A. & Lord, E. M. (2000). Alily stylar pectin is necessary for pollen tube adhesion to an in vitro stylar matrix, *Plant Cell*. 12, 1737.
20. Knox, R. B., Pollen- pistil interaction. In Linskens, H. F. & Heslop-Harrison, J., [eds]. (1984). *Cellular interactions*, 508, Springer- Verlag, Berlin, Heidelberg, NY.
21. Gonzalez, M. U., Coque, M. & Herrero, M. (1996). Pollen pistil interaction in Kiwi fruit, *Amer. J. Bot*., 83, 148.
22. Herrero, M. & Dickinson, H. G. (1980). Pollen tube growth following compatible and incompatible inter-specific pollinations in Petunia hybrida, *Planta*, 148, 217.
23. Kroh, M. (1980). *Nature of intercellular substance of transmitting tissue*. In Loewus, F., [eds]. (1973). Biogenesis of plant cellular wall polysaccharides, 195. Academic Press, New York.
24. Kroh, M. & Helsper, J.P.F.G. (1974). *Transmitting tissue and pollen tube growth. In Linskens*, H.F. [ed], *Fertilization in higher plants*. 167. North Holland Publishing Co., Amsterdam.

25. Michaux, N. (1971). Structure et fonctionement du meristem apical du Pteris cretic L.*Annales des Science Naturelles Botanique*, 11, 17.
26. Gahan, P. B. (1984). *Plant histochemistry and cytochemistry: An introduction*. Academic Press, London.
27. Feder, N. & O' Brien, T. P. (1968). Plant micro technique: some principles and new methods, *Amer. J. Bot*., 55, 123.
28. Gutmann, M. (1993). *Lokalisierung von Polyphenolenin Blattern. Interspezifischer Pfropf kombinationen. Untersuchungen uberden Einflussvon Inkompatibilitat bei Prunus axium L. und Prunus cerasus L. Thesis*. Technischen Universitat Munchen.
29. Mackenzie, J. C., Yoo, B. Y. & Seabrook, J. E. A. (1990). Stigma of Solanum tuberosum CV Shepody, morphology, ultra structure, and secretion, *Amer. J. Bot*., 77, 1111.
30. Dumas, C., Rougier, M., Zandonella, P., Ciampolini, F., Cresti, M. & Pacin, E. (1978). The secretory stigma in Lycopersicum peruvianum, Mill: Ontogenesis and glandular activity, *Protoplasma*. 96, 173.
31. Konar, R. N. & Linskens H. F. (1966). The morphology and anatomy of the stigma of Petunia Hybrida, *Planta*., 71, 356.
32. Konar, R. N. & Linskens, H. F. (1966). The physiology and biochemistry of the stigmatic fluid of Petunia hybrida, *Planta*., 71, 372.
33. Martin, F. W. (1969). Compounds from the stigmas of ten species, *Amer. J. Bot*, 56, 1023.
34. Martin, F. W. (1970). Compound of the stigmatic surface of Zea mays L, *Annals of Bot*., 34, 835.
35. Martin, F. W. (1970). The ultraviolet absorption profile of stigmatic extracts, *New Phyto*l., 69, 425.
36. Martin, F. W. & Telek, L. (1971). The stigmatic secretion of sweet potato, *Amer. J. Bot*., 58, 317.
37. Schou, O. (1984). The dry and wet stigma of Primula obconica: Ultrastructural and cytochemical dimorphisms, *Protoplasma*., 121, 99.
38. Katz, E. (1926). Uber di funktion der narbe beider keimung des pollens, *Flora (Jena),* 20, 243.
39. Motten, A. F. & Antonovics, J. (1992). Determination of outcrossing rate in a predominantly self-fertilizing weed Datura stramonium (Solanaceae), *Amer. J. Bot*., 79, 419.
40. Motten, A. F. & Stome, J. L. (2000). Heritability of stigma position and the effect of stigma-anther separation on outcrossing in a predominantly self fertilizing weed, Datura sramonium (Solanaceae). *Amer. J. Bot*., 87, 339.
41. Sanders, L. C. & Lord, E. M. (1989). Directed movement of latex particles in the gynoecia of three species of flowering plants, *Science*, 243, 1606.
42. Lord, E. M., Walling, L. L. & Jauh, G. Y. (1996). *Cell adhesion in plants and its role in pollination. In membranes: Specialized functions in plants, Smallwood, M., Knox, J.P., and Bowles, D.J*., [eds]. 21. Oxford, UK: Bios Scientific Publishers.
43. Wolters-Arts, Lush, M. W. M. & Mariani, C. (1998). Lipids are required for directional pollen tube growth, *Nature*, 392, 818.
44. Vasil, I. K. (1974). *The histology and physiology of pollen germination and pollen tube growth on the stigma and in the style. In Linskens, H.F., [ed], Fertilization in higher plants,* 105. North Holland Publishing Co., Amsterdam.
45. Mariani, C. & Walters-Arts, M. (2000). Complex Waxes, Plant Cell., 12, p.1495.
46. Arbeloa, A. & Herrero, M. (1987). Germination y difasion proteins del pollen de Melo cotonero, *Itea*., 69, 47.
47. Clifford, S. C. & Owens, S. J. (1990). The stigma, style, and ovarian transmitting tract in the oncidiinae (orchidaceae): morphology developmental anatomy and histochemistry, *Bot Gaz*., 151, 440.
48. Herrero, M. & Arbeloa, A. (1989). Influence of the pistil on pollen tube kinetics in peach (Prunus persica), *Amer. J. Bot*., 76, 1441.

49. Herrero, M. (1998). *Mechanisms in the pistil that regulate gametophytic population in peach (Pianus persica). In. Otta viano,* E., Mulcahy, M., Sari-Gorla, D. L. & Bergamini Mulchahy, G. [eds], *Angiosperm pollen and ovules,* 347. Springer-verlag, New York, NY.
50. 50 Hormaza,J. I. & Herrero, M., Gametophytic competition and selection, In William, E. G., Knox, R. B., and Clark, A. E., [eds]. (1994). *Genetic control of self-incompatibility and reproductive development in flowering plants*, Kluwer Academic Publishers, Netherlands, 372.
51. Wang, Y., Hamburger, M., Gueho, J. & Hostettmann, K. (1989). Antimicrobial flavonoids from Psidia trivernia and their methylated and acetylated derivatives, *Phytochemistry*, 28, 2323.
52. Ruiz- Barba, J. L., Rios-Sanchez, R., Fedriani- Iriso, C., Olias, J. M., Rios, J. L. & Jimenez-Diaz, R. (1990). Bactericidial effect of phenolic compounds from green olives on Lacto bacillus plantarum, *Systematic and Applied Microbiology*, 13, 199.
53. Weidenboerner, M., Hindorf, H., Jha, H. C. & Tsotsonos, P. (1990). Antifungal activity of flavonoids against storage fungi of the genus Aspergillus, Phytochemistry, 29, 1103.
54. Elliger, C. A., Chan, B. C. & Waiss, A. C. (1980). Flavonoids as larval growth inhibitors, *Natur- wissen schaften*, 67, 358.

APPENDIX

Figs. 1-6. Detection of lipid secretions. 1. Longitudinal section of a young stigma in a 15-20 mm long bud. Papillae and secretory cells were filled with small granules stained black with Sudan black B (arrows). This staining indicates their lipidic nature. X 620. Bar = 25 μ m.

2. Stigmatic papillae in a developing stigma after Sudan black B staining. Papilla was covered by a smooth cuticle. The intense color of the cuticle and its thickness at the apex of the papillae indicated accumulation of the lipidic secretion underneath it. The lipidic secretion was also clearly visible in the form of a dark layer beneath the plasmalemma (arrows). X 1600. Bar = 4 μm. 3. Longitudinal section of papillae in a developing stigma; indicating intracellular lipids (small arrows) and early formation of lipidic excretion beneath the ruptured cuticle (arrow heads). Note a lysing stigmatic papilla at left hand side, while keeping intracellular lipids (small arrows). X600. Bar =10μm. 4. Longitudinal section through a young developing stigma stained with Sudan black B, indicating a small amount of secretion in the intercellular space of the secretory cells, (arrow heads). Note the disruption of the thin cuticle between the papillae (small arrow) facilitating the exudate to spread over the stigma surface. X 650. Bar = 20 μm 5. A part of secretory tissue in a mature stigma, stained with Sudan black B, indicating a large amount of lipidic secretion in intercellular spaces (arrows) and its migration to the stigma surface through anticlinal walls, widely separated from each other. X 500. Bar = 20 μm 6. Longitudinal median section through a mature stigma after staining with Sudan black B, indicating two distinct regions with different secretory characteristics: 1) Superficial zone constituted of lipid secreting cells. Note the intercellular accumulation of lipids stained black. 2) Internal zone constituted of transmitting tissue cells and non stainable with Sudan black. X 80. Bar = 100 μm.

Figs. 7-10. Detection of carbohydrates. 7. Section of secretory cells of a pollinated stigma. The intercellular substance reacted weakly with PAS stain. Note deeply stained pollen grains and pollen tubes growing between secretory cells (arrows). X 250. Bar = 50 μm. 8. Longitudinal section of transmitting tissue in 40-45 mm long bud stained with PAS method; indicating thickened walls of this tissue, strongly stained for carbohydrates (arrows). X1600. Bar = 10 μm. 9. Longitudinal section in the style in a 20-25 mm long bud stained with PAS method. Parenchyma cells were rich in starch reserves (single arrows). Note accumulation of starch in dorsal parenchyma. Other possible storage

bodies (double arrow) did not stain as starch or lipids. X 200. Bar = 50 µm. 10. Median longitudinal section in a 25-30 mm long bud stained with PAS, indicating the end part of the style and a portion of the stigma. Note the absence of starch reserves in the parenchyma cells below the stigma. X 160. Bar=100µm.

Figs. 11-14. Detection of proteins and polyphenols. 11. Cross section of secretory cells of a mature stigma stained with Coomasie brilliant blue; indicating the absence of staining in intercellular substance (asterisks). Note the intravacuolar globules stained positively (arrows). X 625. Bar= 20 µm. 12. Cross section of secretory cells of a mature stigma stained with Toluidine blue; indicating the absence of staining in intercellular substance for polyphenols. Note the intravacuolar globules stained positively (arrows).

X 625. Bar = 20 µm. 13. Cross section of secretory cells of a mature stigma stained with Safranin; to reveal polyphenols. Note the intravacuolar globules stained positively (arrows). X 1000. Bar = 10 µm. 14. Cross section of secretory cells of a mature stigma stained with Safranin. Note the intensely stained globules (arrows) released out of the cells after autolysis. X 1600. Bar = 10µ m