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Structure and cytochemistry of the pistil in *Arachis hypogaea*

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Abstract. In *Arachis hypogaea* (Papilionoideae, Leguminosae), the stigma is of the dry papillate type. The papillae are multicellular and multiseriate. They are covered with a thin lining of pellicle which responds for proteins, non-specific esterases and acid phosphatases. The style is 3–6 cm long and hollow throughout its length. The stylar canal is bordered by a layer of canal cells. The canal cells in most of the stylar region are not glandular; they are vacuolate with scant cytoplasm. The canal cells at the base of the style, however, are glandular with dense cytoplasm and prominent nuclei. The structural features of the pistil of *Arachis* are discussed with those of other Papilionoideae.

Keywords. *Arachis hypogaea*; groundnut; legume; pistil; pollen-pistil interaction; stigma; style.

1. Introduction

Studies on the details of the pistil and of pollen-pistil interaction are important not only in understanding the biology of sexual reproduction but also in applied aspects of fruit- and seed-set (see Shivanna and Johri 1985). Such basic data are also useful in manipulating hybridization barriers. Studies on these aspects on members of Leguminosae (Papilionoideae) have been initiated only recently and are, so far, confined to *Vigna* (Ghosh and Shivanna 1982), *Trifolium* (Heslop-Harrison Y and Heslop-Harrison J 1982; Heslop-Harrison J and Heslop-Harrison Y 1982), *Cicer* (Malti and Shivanna 1983), *Crotalaria* (Malti and Shivanna 1984), *Vicia* (Lord and Heslop-Harrison 1984) and *Phaseolus* (Heslop-Harrison J and Heslop-Harrison Y 1984).

Arachis hypogaea is one of the most important legumes in India. Although intensive studies are being carried out on interspecific hybridization (Sastri and Moss 1982; see also Sastri 1984) in this taxon, no details are available on the structure of the pistil and on pollen-pistil interaction. This paper reports anatomical and cytochemical details of the pistil in *Arachis hypogaea*.

2. Material and methods

Plants of *Arachis hypogaea* Linn. var. TMV-2 (an annual, branched, prostrate type), grown under field conditions were used in our studies. For cytochemical studies on the stigma, flower buds at 3 developmental stages—2 days before anthesis, 1 day before anthesis and on the day of anthesis—were used. Stigma surface proteins were localized with 0·25% coomassie brilliant blue R in 7% acetic acid (Heslop-Harrison *et al* 1974). Non-specific esterases were localized using α-napthyl acetate as a substrate in a coupling reaction with fast blue B (Mattsson *et al* 1974) and acid phosphatases with α-naphthyl acid phosphate as a substrate in a coupling reaction with fast garnet GBC

(Scandalios 1969). The cuticle was localized with 0·02 % aqueous auramine 0 (Heslop-Harrison 1977). Lipoidal material on the stigma was stained with sudan black B (Jensen 1962).

Anatomical studies of pistils were carried out using semithin sections (2 μm). As the pistil in *Arachis* is long (3–6 cm) about 5 mm segments from each of the following 4 levels were used for fixation and sectioning: (i) the stigma and a few millimeter of the subjacent style, (ii) middle region of the staminal tube, (iii) middle region of the calyx tube, and (iv) the lower most part of the style together with the upper part of the ovary.

Pistils were fixed in 10 % aqueous acrolein for 24 hr at 0° C and dehydrated through a 2-methoxyethanol—ethanol—*n*–propanol—*n*–butanol series (Feder and O'Brien 1968). The dehydrated material was infiltrated and embedded in JB_4 resin (Polysciences, USA). Sections were cut at 2 μm thickness and stained with 0·1 % toluidine blue (Merck) in 0·1 M acetate buffer, pH 4·5 (Feder and O'Brien 1968); 0·25 % coomassie brilliant blue R (Weber and Osborn 1975) for proteins, periodic acid-Schiff's reagent (PAS, McGuckin and McKenzie 1958; Feder and O'Brien 1968) for insoluble polysaccharides; and 0·02 % aqueous auramine 0 (Heslop-Harrison 1977) for cuticle.

3. Observations

The flower is subsessile. The calyx forms a long tube, called the hypanthium, enclosing the ovary and most of the proximal part of the style, and appears very much like a pedicel (figure 1A, B). There are 10 stamens of which 8 bear fertile and 2 sterile anthers. Of the 8 fertile anthers, 4 are linear and the remaining 4 round, arranged alternately in a ring. Anther filaments arise from the rim of the calyx tube. The lower parts of anther filaments are fused forming a sheath around the style, above the calyx tube, while the terminal parts of the filaments are free.

The pistil is 3–6 cm long and most of it is formed by the style. The ovary is small and inconspicuous at the base of the style. About two thirds of the style is enclosed by calyx tube. The style is curved at right angles to the long axis at the tip of the calyx tube and again at the tip of the staminal tube (figure 1B). The stigma is terminal and club shaped (figure 1C). Non-receptive hairs are present on the upper part of the style.

The stigma is of the dry, papillate type (Heslop-Harrison and Shivanna 1977) without any visible exudate. The papillae are globose, multicellular and multiseriate (figure 1F). The papillae show a thin lining of proteinaceous pellicle which could be localized following staining with coomassie blue (figure 1D). The pellicle occurs on the surface of the papillae as a continuous layer. The pellicle is present in all the stages of buds studied; however, there is an increase in the intensity of staining with the maturity of the pistil.

The pellicle also responds for non-specific esterases and acid phosphatases. While esterases form a smooth and continuous layer (figure 1E) phosphatases are distributed in the form of granules. The activity of both the enzymes increases with the maturity of pistils.

The stigma surface as well as the contents of the stigmatic papillae stain intensely with sudan black B. Small globules of lipoidal material are also present in the papillae. The cuticle of the papillae is extremely thin and shows very faint fluorescence with auramine 0.

The stigma is solid with a core of transmitting tissue. The style is hollow throughout its length and is traversed by a canal which is continuous with the ovarian cavity. The

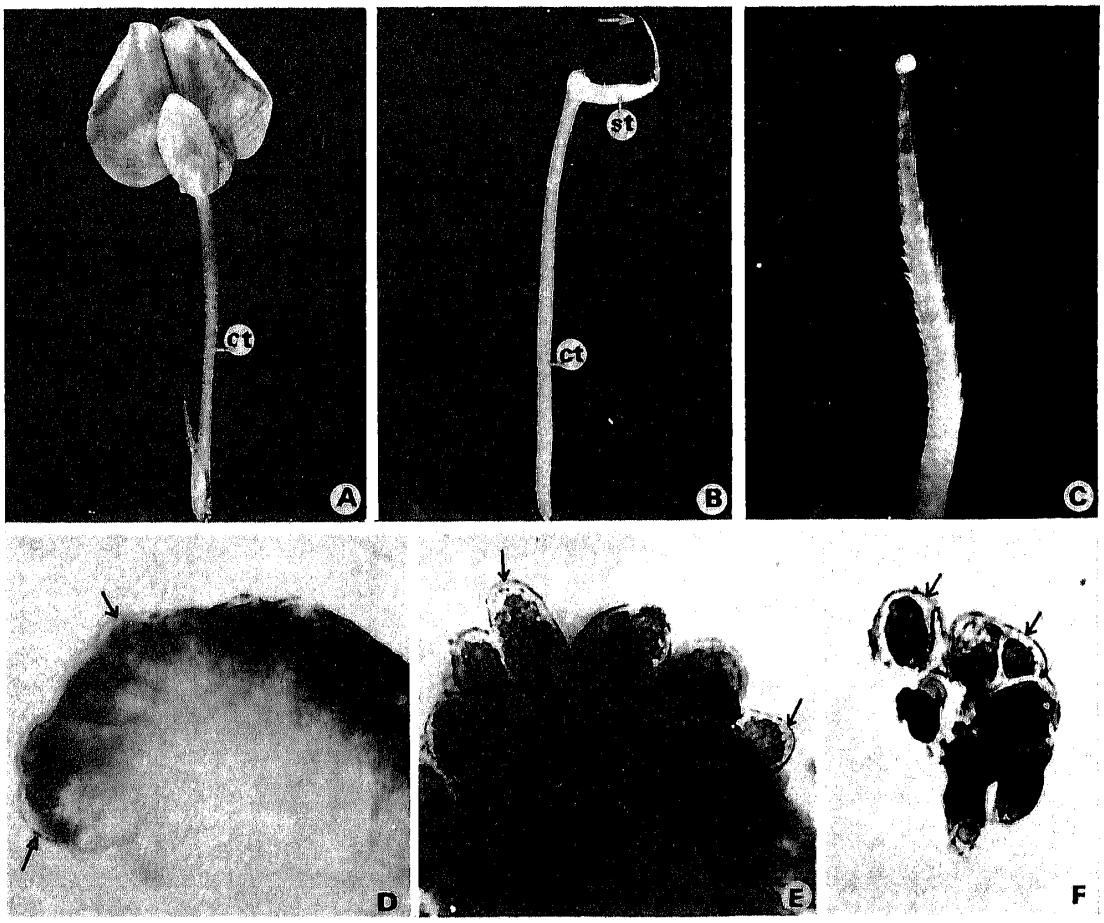

Figure 1. **A.** Front view of a flower soon after anthesis. Observe long calyx tube (ct) enclosing the lower part of the style. **B.** Entire pistil from a flower on the day of anthesis. Ovary and most of the proximal part of the style are enclosed in calyx tube (ct) and staminal tube (st). Arrow points to the stigma. **C.** Stigma and a part of the subjacent style at higher magnification. Stylar hairs and club shaped stigma are obvious. **D.** Wholemount of the stigma stained with coomassie blue. Coomassie blue positive pellicle is clearly visible at many places (arrows). **E.** A part of the stigma following localization of non-specific esterases. Apart from cytoplasmic esterases, pellicle of the papillae also show esterase activity (arrows). **F.** A few papillae from E to show multicellular nature of the papillae and pellicle (arrows).

stigmatic papillae converge at the base of the stigma and form a strand of transmitting tissue. Transections of the stigma at the base show an epidermal layer and cells of the transmitting tissue with large intercellular spaces. There is no cavity at this level (figure 2A).

Transections below the stigma show the initiation of a small stylar cavity which is not bordered by a well-demarcated layer of canal cells (figure 2B). The stylar cavity gradually widens toward the lower part of the style (figures 2C–F) and gets surrounded by a well-demarcated layer of cells, canal cells (figures 2E, F). The cortical region becomes distinguishable. The stylar cavity is not bordered by cuticle as revealed by auramine 0 preparations (figure 2D).

The canal cells are not glandular. They are vacuolate with scant cytoplasm and thus are very much similar to the cortical cells. The cell wall around the canal cells is

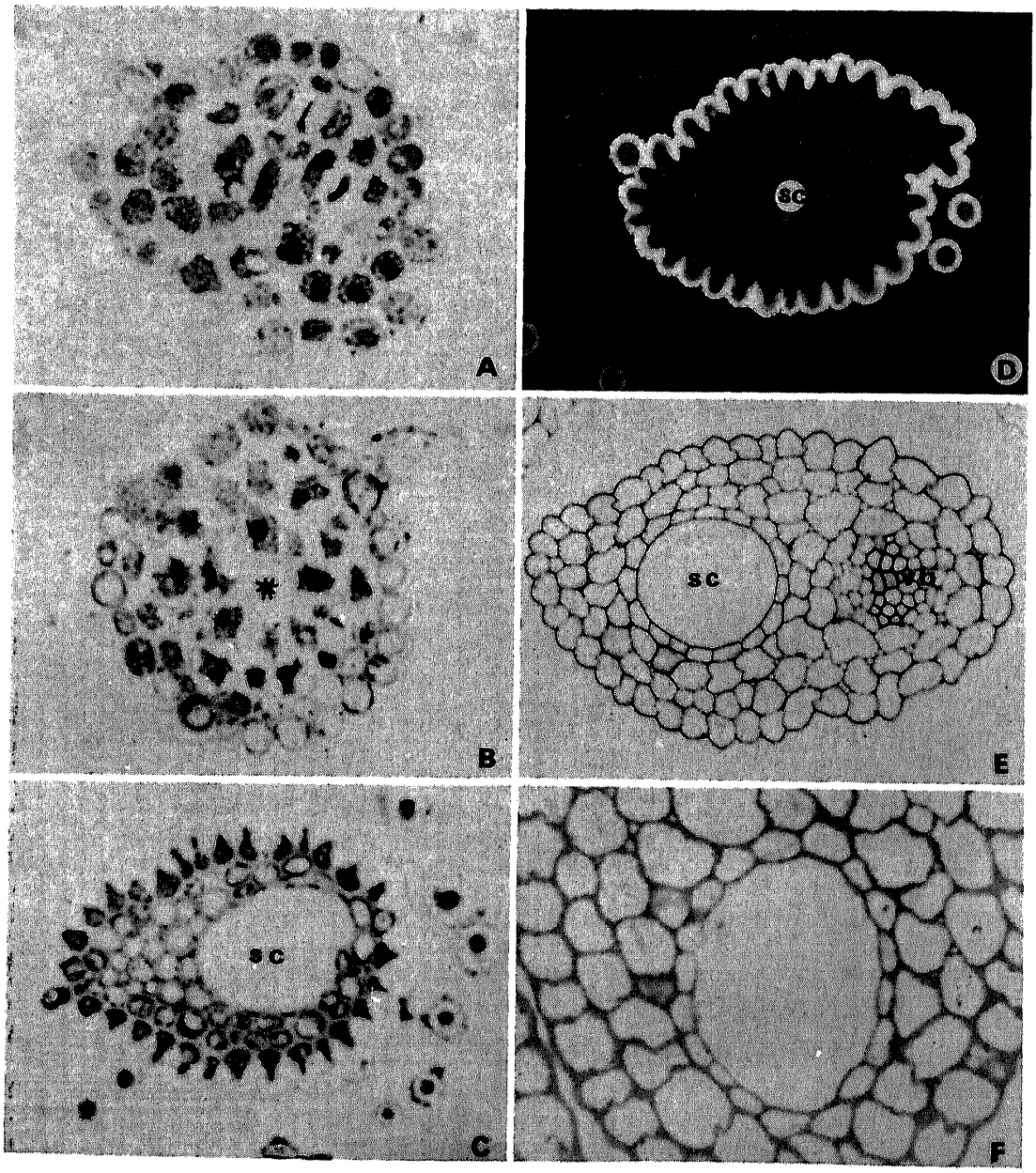

Figure 2. **A.** TS of the stigma at the base stained with toluidine blue to show the cells of the transmitting tissue with large intercellular spaces formed by the converging stigmatic papillae. **B.** TS of style just below the stigma. A small cavity has appeared at the centre of the transmitting tissue (asterisk). **C.** TS style further down the stigma. The stylar canal (sc) has enlarged and is not lined with well-demarcated canal cells. **D.** TS style a few mm below the stigma stained with auramine 0. The stylar canal is not lined with a cuticle; a thick cuticle is obvious on the outer surface of epidermal cells. **E.** TS middle portion of style stained with PAS reagent. Stylar canal is lined with well-demarcated layer of canal cells. Vb–vascular bundle. **F.** Similar to E but stained with coomassie blue. Canal cells are non-glandular and resemble cortical cells.

continuous as revealed by PAS staining (figure 2E). It does not show any thickening of the inner tangential wall facing the canal.

The cortical cells of the style are loosely arranged with conspicuous intercellular spaces. The epidermal layer is lined externally with a layer of thick cuticle (figure 2D).

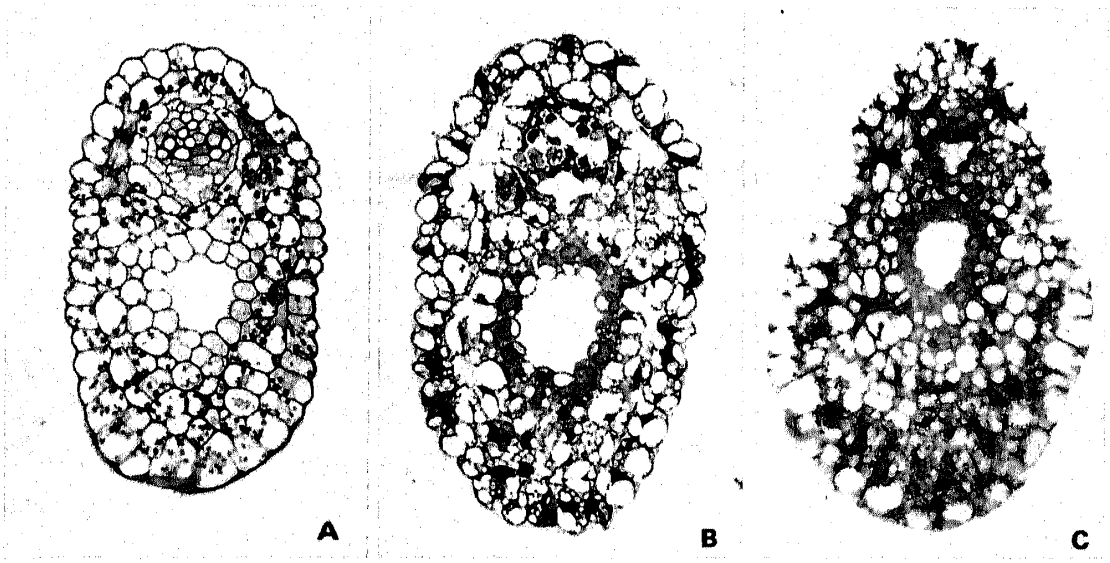

Figure 3. Structure of the pistil in the transitory zone (lower part of the style and upper part of the ovary). **A.** TS lower part of the style stained with PAS. The starch grains are clear in the cortical and epidermal cells. The canal cells are large and are free from starch grains. **B.** As in A, but further down, stained with coomassie blue. The canal cells are filled with dense cytoplasm and prominent nuclei. **C.** TS style at the tip of the ovary, stained with coomassie blue. The canal cells are completely filled with dense cytoplasm. A few cells surrounding the layer of canal cells are also prominent.

Non-receptive hairs on the style are also lined with a layer of thick cuticle. The style has a dorsal vascular bundle traversing the whole length which continues into the ovary. The structure of the style is basically similar along almost the whole length.

The lower part of the style enlarges and gradually merges with the ovary. The details of this zone are distinct from the rest of the style. The canal cells in this region are large, glandular with dense cytoplasm and prominent nuclei (figures 3A–C). A few cortical cells surrounding the layer of canal cells also become prominent with dense cytoplasm. As the ovary is approached, the glandular cells increase in size and the canal eventually leads into the ovarian cavity.

Both in the lower part of the style and in the ovary, the cells of the cortex contain abundant starch grains (figure 3A). In the cells of stigma and upper part of the style, however, starch grains are almost completely absent.

Cytochemical techniques do not reveal any contents in the stylar cavity. As these studies are confined to the fixed and processed material, the possibility of removal of the stylar contents during processing is not ruled out.

4. Discussion

Studies conducted so far on the details of the pistil of many taxa belonging to Papilionoideae of Leguminosae have shown considerable variation in finer details, although they are similar in basic features. The stigma is generally wet and papillate (see Heslop-Harrison and Shivanna 1977). The exudate may be copious as in *Vigna* and *Cajanus* (Ghosh and Shivanna 1982), *Phaseolus* (Lord and Webster 1979) and *Trifolium* (Heslop-Harrison J and Heslop-Harrison Y 1982) or scanty as in *Crotalaria* (Malti and

Shivanna 1983). The cells of the stigma are secretory. In taxa which produce copious exudate, the secretion appears on the stigma surface only after the cuticle is disrupted (Heslop-Harrison J and Heslop-Harrison Y 1984). The pistil of *Arachis hypogaea*, although basically resembling that of other Papilionaceous taxa, shows many important variations. Unlike all other taxa so far studied, there is no visible exudate on the stigma of *A. hypogaea*. The papillae are covered by a layer of pellicle similar to those of other taxa with a dry stigma. Also, in contrast to unicellular papillae of other taxa, stigmatic papillae of *A. hypogaea* are multicellular and multiseriate.

The style, although hollow in all Papilionaceous taxa, shows many variations. In *Vigna unguiculata* (Ghosh and Shivanna 1982) the upper part of the style is typically solid with a strand of transmitting tissue. Further down, an irregular cavity appears in the centre of the transmitting tissue by dissolution of the cells. The cavity gradually enlarges towards the lower part of the style and eventually the transmitting tissue becomes confined to 1–3 layers bordering the cavity. This feature is maintained throughout the lower part of the style.

In many other taxa studied (*Trifolium*, Heslop-Harrison Y and Heslop-Harrison J 1982; Heslop-Harrison J and Heslop-Harrison Y 1982; *Cajanus*, Ghosh and Shivanna 1982; *Cicer*, Malti and Shivanna 1983; and *Crotalaria*, Malti and Shivanna 1984), almost the entire style is hollow. The stylar canal is bordered by one or a few layers of glandular cells, the canal cells, which are densely cytoplasmic. In *Arachis* also the style is hollow throughout its length. One of the important structural differences of the style in *Arachis*, when compared to other taxa, is the absence of glandular cells bordering the stylar canal. The canal cells are vacuolate with scant cytoplasm and resemble cortical cells. Extracellular proteinaceous material on the surface of the inner tangential wall of the canal cells appears to be absent. However, canal cells in the upper part of the ovary become glandular. They are densely cytoplasmic with large nuclei.

Studies conducted so far on the details of the pistil in different groups of plants, have invariably shown the presence of extracellular secretion products in the path of the pollen tubes in the style (either in the stylar canal or in the intercellular spaces of the transmitting tissue). Much evidences suggest that these extracellular components, apart from providing nutrition to the growing pollen tubes, are involved in incompatibility responses (see Shivanna 1979, 1982).

In both *Trifolium* (Heslop-Harrison J and Heslop-Harrison Y 1982) and *Crotalaria* (Malti and Shivanna 1984) in which pollen tubes have been traced in the stylar canal throughout its length, the stylar canal contains extracellular components either in the stylar-fluid or as a layer on the inner surface of canal cells. Post-pollination secretion also seems to occur as indicated by the collapse of the surrounding canal cells. Based on fluorescent microscopic studies of wholemounts of pistils (stained with aniline blue), Hawkins and Evans (1973) reported that in *Phaseolus coccineus*, pollen tubes grow in the vicinity of the vascular tissue of the style. They suggested that pollen tubes depend on the phloem for their nutrition. Structural details of the pistil of *Phaseolus* are not available.

As pointed out earlier, in *Arachis*, except in the region just below the stigma and the upper part of the ovary, there is no visible secretion product in the stylar cavity. There is not much scope for post-pollination secretion, as the canal is not lined by glandular cells. It is quite possible that the pollen tubes after traversing a short distance in the stylar canal just below the stigma, may grow in the vicinity of the vascular bundle as has been reported in *P. coccineus*. This aspect needs to be studied.

References

Feder N and O'Brien T P 1968 Plant microtechnique: some principles and new methods; *Am. J. Bot.* **55** 123–142

Ghosh S and Shivanna K R 1982 Anatomical and cytochemical studies on the stigma and style in some legumes; *Bot. Gaz.* **143** 311–319

Hawkins G F and Evans A M 1973 Elucidating the behaviour of pollen tubes in intra- and inter-specific pollinations in *Phaseolus vulgaris* L. and *P. coccineus* Lam.; *Euphytica* **22** 378–385

Heslop-Harrison J and Heslop-Harrison Y 1982 Pollen-stigma interaction in the Leguminosae: The organization of the stigma in *Trifolium pratense* L.; *Ann. Bot.* **51** 571–583

Heslop-Harrison J and Heslop-Harrison Y 1984 Stigma organization and control of fertilization in *Phaseolus*; in *Proceedings Eucarpia Meeting on Phaseolus Bean Breeding* (ed.) Reimann-Philipp pp 88–96

Heslop-Harrison J, Knox R B and Heslop-Harrison Y 1974 Pollenwall proteins: exine held fractions associated with the incompatibility response in Cruciferae; *Theor. Appl. Genet.* **44** 133–137

Heslop-Harrison Y 1977 The pollen-stigma interaction: Pollen tube penetration in *Crocus; Ann. Bot.* **41** 913–922

Heslop-Harrison Y and Heslop-Harrison J 1982 Pollen stigma interaction in the Leguminosae: the secretory system of the style in *Trifolium pratense* L.; *Ann. Bot.* **50** 635–645

Heslop-Harrison Y and Shivanna K R 1977 The receptive surface of the angiosperm stigma; *Ann. Bot.* **41** 1233–1258

Jensen W A 1962 *Botanical Histochemistry* (London: W H Freeman and Co)

Lord E M and Heslop-Harrison Y 1984 Pollen-stigma interaction in the Leguminosae: Stigma organization and breeding system in *Vicia faba* L.; *Ann. Bot.* **54** 827–836

Lord E M and Webster B D 1979 The stigmatic exudate of *Phaseolus vulgaris* L.; *Bot. Gaz.* **140** 266–271

Mattsson O, Knox R B, Heslop-Harrison J and Heslop-Harrison Y 1974 Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions; *Nature (London)* **247** 298–300

McGuckin W F and McKenzie B F 1958 An improved periodic acid Fuchsin sulfite staining method for evaluation of glycoproteins; *Clin. Chem.* **4** 476–483

Malti and Shivanna K R 1983 Pollen-pistil interaction in chickpea; *Int. Chickpea Newslett.* No. 9 10–11

Malti and Shivanna K R 1984 Structure and cytochemistry of the pistil of *Crotalaria retusa* L.; *Proc. Indian Nat. Sci. Acad.* **B50** 92–102

Sastri D C 1984 Incompatibility in angiosperms: Significance in crop improvement; *Adv. Appl. Biol.* **10** 71–111

Sastri D C and Moss J P 1982 Effect of growth regulators on incompatible crosses in the genus *Arachis* L.; *J. Exp. Bot.* **33** 1293–1301

Scandalios J G 1969 Genetic control of multiple molecular forms of enzymes in plants: a review; *Biochem. Genet.* **3** 37–79

Shivanna K R 1979 Recognition and rejection phenomena during pollen-pistil interaction; *Proc. Indian Acad. Sci. (Plant Sci.)* **88** 115–141

Shivanna K R 1982 Pollen-pistil interaction and control of fertilization; in *Experimental Embryology of Vascular Plants* (ed) B M Johri (Berlin: Springer-Verlag) pp 131–174

Shivanna K R and Johri B M 1985 *The Angiosperm Pollen: Structure and Function* (New Delhi: Wiley Eastern)

Weber K and Osborn M 1975 Proteins and sodium dodecyl sulfate: molecular weight determination on polyacrylamide gels and related procedures; in *The Proteins* (eds) H Neurath and R L Hill (New York: Academic Press) **1** 179–223