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Pollen-pistil Interaction in a Non-pseudogamous Apomict, *Commiphora wightii*

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Structural and cytochemical details of the pistil and the interaction of pollen and pistil were studied in a non-pseudogamous apomict, *Commiphora wightii*. The anthers in the male and bisexual flowers produce functional pollen grains. The stigma is of the wet and papillate type. The style is typically solid with two strands of transmitting tissue that traverse the entire length of the style. There is a marked reduction in the area occupied by the transmitting tissue from the stigma to the base of the style. The cells of the transmitting tissue are isodiametric in transverse as well as longitudinal section and do not form longitudinal files of elongated cells as reported for other taxa. Proteins could not be localized in the intercellular matrix. Although pollen grains germinate on the stigma, pollen tubes do not grow beyond the proximal one third of the style. Changed orientation of the cells of the transmitting tissue and absence of proteins in the intercellular matrix could account for the failure of the pistil to support pollen growth.

Key words: Guggul, pollen-pistil interaction, non-pseudogamous apomict, *Commiphora wightii*, transmitting tissue.

INTRODUCTION

Extensive investigations have been carried out to elucidate the structural and functional aspects of pollen-pistil interaction in a number of sexually reproducing flowering plants (Heslop-Harrison and Shivanna, 1977; Tilton and Horner, 1980; Heslop-Harrison, Heslop-Harrison and Shivanna, 1981; Dickinson, Moriarty and Lawson, 1982; Lord and Heslop-Harrison, 1984; Shivanna and Johri, 1985; Knox, Williams and Dumas, 1986; Owens, 1989; Cresti, Blackmore and Van Went, 1992; Sanders and Lord, 1992; Shivanna, Cresti and Ciampolini, 1997). However, there are very few studies on the structural details of the pistil and of pollen-pistil interaction in apomictic species (Richards, 1990).

Our recent studies of the breeding system of *Commiphora wightii* (Arnott) Bhandari (Burseraceae) showed that the species is a sporophytic apomict showing nucellar polyembryony. The apomixis is independent of pollination stimulus i.e. non-pseudogamous (Gupta, Shivanna and Mohan Ram, 1996) although the plants produce morphologically normal pollen and pistils. The structure of the pistil and details of pollen-pistil interaction in this species are described in this paper.

MATERIALS AND METHODS

A population of guggul plants raised at the Central Arid Zone Research Institute (CAZRI), Jodhpur was used for detailed investigation. This population is made up of 55 female, two male and one andromonoecious plants (Gupta *et al*., 1996). Comparative studies were also made on natural populations growing at Indroka village (Jodhpur) and those maintained in the Department of Botany garden, University of Delhi.

Stigma surface proteins were localized by staining with Coomassie brilliant blue R (Gurr) (Heslop-Harrison, Knox and Heslop-Harrison, 1974). Non-specific esterases were localized on the stigma surface using α-naphthyl acetate as a substrate and fast blue B as a coupling reagent (Mattsson *et al*., 1974). Pollen viability was estimated on the basis of FCR (fluorochromatic reaction) test (Heslop-Harrison and Heslop-Harrison, 1970). Flowering twigs were brought from CAZRI to the laboratory in Delhi and pollen grains from freshly dehisced anthers were used to test viability.

Pollinations were made using fresh pollen grains from male as well as bisexual flowers onto stigmas of bagged intact female flowers as well as of excised pistils implanted on 0·8% agar set in Petri dishes. The pistils were fixed 6–48 h after pollination in acetic-alcohol (3:1) and pollen germination and pollen tube growth were studied using the aniline blue fluorescence technique (Linskens and Esser, 1957; Shivanna and Rangaswamy, 1992).

For anatomical studies, male and female flowers were fixed in 10% acrolein and processed for resin embedding (Feder and O'Brien, 1968). Semi-thin sections (2–3 μm) were cut with glass knives and were used for structural and cytochemical studies. Sections were stained with the metachromatic stain toluidine blue O (Feder and O'Brien, 1968). Sections were also treated with cytochemical stains for localization of polysaccharides (PAS; McGuckin and McKenzie, 1958), proteins (Coomassie brilliant blue; Bradford, 1976), pectins (alcian blue; Heslop-Harrison, 1979), lipids (Sudan III; Jensen, 1962) and cuticle (auramine O; Heslop-Harrison, 1977).

For scanning electron microscopic studies, freshly dehisced anthers and pistils at various developmental stages

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were fixed in glutaraldehyde (3%), dehydrated in an ascending series of acetone, critical point dried and mounted on aluminium stubs before being sputter coated with gold. The materials were observed in a Philips EM 501 scanning electron microscope.

RESULTS

The flowers were mostly unisexual. The female flowers bear a superior ovary raised on a nectary disc with staminodes arranged in two whorls of four. Sections of staminodes showed early degeneration of the sporogenous tissue. The male flowers bear stamens arranged in two whorls of four and a centrally located minute pistillode. The bisexual flowers have fertile stamens as well as a fully developed pistil.

Pollen grains

Pollen grain development is normal in male and bisexual flowers. They are isopolar, trizonocolporate with tectate exine showing reticulate ornamentation (Fig. 1A). Lipids are the reserve material in pollen grains. The FCR test of fresh pollen showed 50% viability. A small proportion (14%) of pollen grains retained viability even after 8 d of storage under laboratory conditions.

The pistil

The pistil is clearly demarcated into the stigma, style and ovary. The stigma is bilobed (Fig. 1B), papillate and is devoid of any exudate prior to anthesis. The papillae are short and compactly arranged. The stigma becomes wet following anthesis. By 12 h after anthesis, the exudate becomes copious and covers the entire surface of the stigma (Fig. 1C). The stigma surface stains for proteins. Non-specific esterases were also localized on the stigma surface. Maximum esterase activity was observed on the stigma 12 h after anthesis.

The style is solid with two separate strands of transmitting tissue corresponding to the two lobes of the stigma (Figs 2 and 3A–C). There is a marked decrease in the area occupied by the transmitting tissue strands from the stigma to the base of the style (Figs 2 and 3A–C, Table 1). At the top of the style, just below the stigma, each strand is made up of 65 ± 3 cells in cross section (Fig. 3A). At the base of the style each strand consists of 10 ± 2 cells (Fig. 3B and C, Table 1). A number of gum-resin canals are also associated with the vascular bundles (Fig. 3A–C).

In transverse section the cells of the transmitting tissue are isodiametric (Fig. 3A–C) with conspicuous intercellular spaces filled with extracellular matrix. The matrix stains for carbohydrates (Fig. 4A), pectinaceous substances and lipids. However, no proteins could be localized in the intercellular matrix through Coomassie blue staining (Fig. 4B). The cells of the transmitting tissue are also isodiametric in longitudinal section and do not form longitudinal files of elongated narrow cells as reported in other solid-styled species (Fig. 3D and E).

Pollen-pistil interaction

Studies conducted to assess stigma receptivity showed that maximum pollen adhesion (85%) and pollen germination (22·5%) occurred in stigmas pollinated 12 h after anthesis (Table 2). However, cleared pistil preparations (6 h after pollination) showed that most of the pollen tubes were restricted to the stigma only (Table 2). The pollen tubes were thick with callose deposition throughout their length and were often branched. Only a few tubes penetrated the stigmatic tissue. Pollen tubes were never observed below the topmost third of the style (Fig. 4C and D).

To confirm the inability of pollen tubes to reach the base of the style, a large number of pollinations were carried out *in vivo* on pistils 12 h after anthesis. These pistils were examined 6–48 h after pollination for pollen germination and tube growth. Out of a total of 315 hand-pollinated pistils surveyed, only five showed one or two pollen tubes in the upper part of style just below the stigma and none of the pistils showed pollen tubes in the lower part of the style. The results were similar in selfed (in bisexual flowers of andromonoecious individuals) as well as cross-pollinated pistils.

DISCUSSION

Previous reports of pollen development in *Commiphora wightii* have indicated that the sporogenous tissue degenerates prematurely (Davis, 1966). However, the present study has established that the male and andromonoecious plants have normal pollen development and bear viable pollen. It is possible that the earlier findings on degeneration of sporogenous tissue were based on examination of staminodes in female flowers. Most features of the pistil in *Commiphora wightii* are comparable to those in a large number of sexually reproducing angiosperm species. The stigma is of wet papillate type. The stigma surface responds to non-specific esterases as is commonly noted in other stigmas (Heslop-Harrison and Shivanna, 1977). The style is solid with two strands of transmitting tissue. An interesting feature of the transmitting tissue is its marked reduction in size from the stigma to the base of the style. Although such a reduction has been reported in a few other systems (Owens, 1989; Herscovitch and Martin, 1989), its significance in pollen-pistil interaction is not clearly understood.

The histological details of the transmitting tissue in *Commiphora wightii* differ from that of sexually reproducing plants. In almost all solid-styled systems investigated to date, the cells of the transmitting tissue are loosely arranged with a conspicuous extracellular matrix, and form files of elongated narrow cells with their transverse walls traversed by plasmodesmata (Sassen, 1974; Heslop-Harrison and Heslop-Harrison, 1982; Ghosh and Shivanna, 1984; Heslop-Harrison, Heslop-Harrison and Roger, 1985). The extracellular matrix provides a continuous path for the growth of pollen tubes along the style. This arrangement of cells in the style is believed to provide a path of least resistance to the growing pollen tubes and thus guide the pollen tubes towards the base of the style without the need for any chemotropic gradient (Heslop-Harrison and Heslop-Harrison, 1986). In *Commiphora wightii*, however, the cells

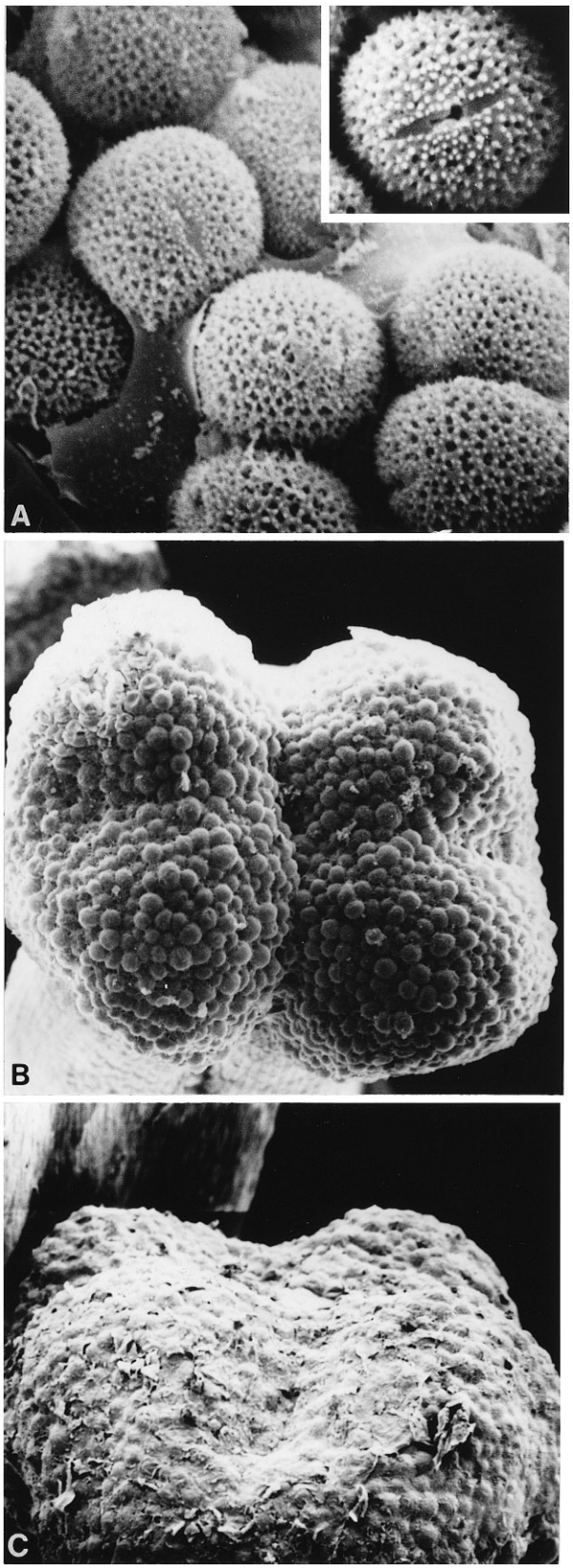

FIG. 1. Scanning electron micrographs of pollen grains and stigma A, Pollen grains showing exine ornamentation; colporate nature of the pollen is clear in the inset. × 816. B, Stigma has hardly any exudate on the day of anthesis. The outlines of papillae are clearly visible. × 200. C, Stigma 1 d after anthesis, with a copious amount of exudate covering the surface. × 200.

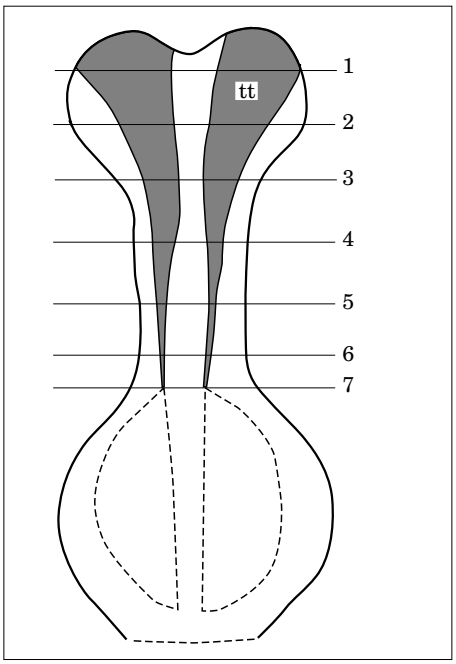

FIG. 2. Diagrammatic representation of the transmitting tissue as seen in a longitudinal section of the pistil. Note the reduction in the extent of transmitting tissue from the stigma to the base of the style. Numbers 1 to 7 represent levels of style cut transversely to obtain histological details presented in Table 1 and Fig. 3.

are isodiametric in both transverse and longitudinal sections; they do not form files of elongated narrow cells and do not provide a continuous path of intercellular matrix for pollen tube growth.

Another variation in the structural features of the style in *Commiphora wightii* is in the composition of the extracellular matrix. Proteins generally form a major component of the extracellular matrix in all the solid-styled species (Heslop-Harrison and Heslop-Harrison, 1982; Knox, 1984; Shivanna and Johri, 1985; Lord and Sanders, 1992; Shivanna *et al*., 1997). In recent years several authors have suggested that extracellular proteins play an important role in pollen tube growth (Wang, Wu and Cheung, 1993; Wu, Wang and Cheung, 1995; Gonzalez, Coque and Herrero, 1996). In *Nicotiana*, extracellular glycoproteins which are expressed only in the transmitting tissue (transmitting tissue specific proteins or TTS proteins), have been identified and characterized (Chen, Mau and Clarke, 1993; Cheung *et al*., 1993). There is evidence that these proteins are directly involved in pollen-tube growth and nutrition (Wang *et al*., 1993; Wu *et al*., 1995). The number of pollen tubes that grow through the style of a mutant deficient in TTS proteins is significantly reduced (Wu *et al*., 1995). Our attempts to localize proteins in the intercellular matrix of the transmitting tissue of *Commiphora wightii* have been unsuccessful.

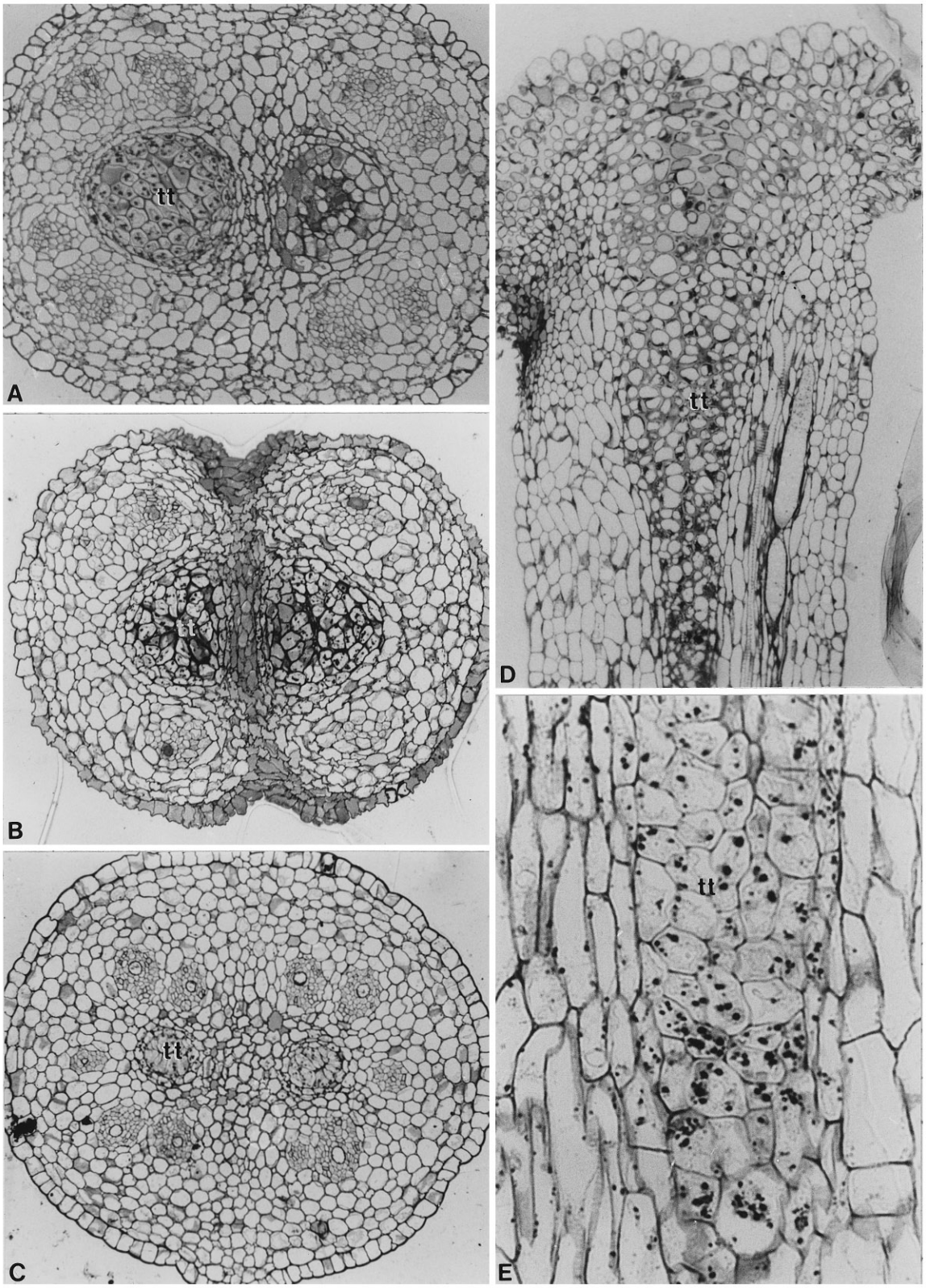

FIG. 3. Structure of the style. A–C, Transverse sections of style at levels 2 (×142), 3 (×140) and 7 (×140), respectively. The marked decrease in the number of cells and area occupied by the transmitting tissue (tt) in B and C is obvious. D and E, Longitudinal sections of stigma and style in the upper (D, ×160) and lower (E, ×180) parts. The cells of the transmitting tissue (tt) are isodiametric and do not form files of elongated and narrow cells.

TABLE 1. *Area occupied by the transmitting tissue at different levels in the style*

Level of style	Area (in 10^3 μm^2) of entire style section	Area (in 10^3 μm^2) of single transmitting strand	Area of the style occupied by both transmitting strands (%)
1	148 ± 13·5	31·5 ± 3·7	42·00
2	118 ± 4·6	24·1 ± 2·0	40·61
3	51 ± 3·2	17·7 ± 2·2	44·00
4	73 ± 3·7	7·2 ± 1·2	19·78
5	106 ± 3·4	5·5 ± 1·0	10·51
6	104 ± 6·4	3·4 ± 2·2	6·46
7	144 ± 18·4	1·4 ± 0·3	0·99

$n = 10$.
Style levels 1–7 represent positions marked in Fig. 2.

TABLE 2. *Stigma receptivity: results of pollinations of pistils implanted in petri dishes*

Stage of flower at pollination	% Pistils showing pollen adhesion	Mean no. of pollen adhering to stigma	Pollen germination (%)	Extent of pollen tube growth
24 h before anthesis	0	0	0	—
12 h before anthesis	0	0	0	—
At anthesis	20	4·2	0	—
12 h after anthesis	85	12·15	22·5	Stigma/style
24 h after anthesis	80	9·2	17·25	Stigma
48 h after anthesis	52	4·7	5	Stigma

$n = 20$, 6 h after pollination.

It is therefore inferred that the proteins concerned are either absent or present in extremely small amounts.

Investigations of pollen germination and pollen tube growth in *Commiphora wightii* using more than 300 pistils have conclusively shown that pollen tubes do not grow through the style. Although many pollen tubes enter the stigma they are mostly confined to the tissues of the stigma, and only a few at most grow for a short distance in the style. Furthermore, pollen tubes show abnormalities, namely a

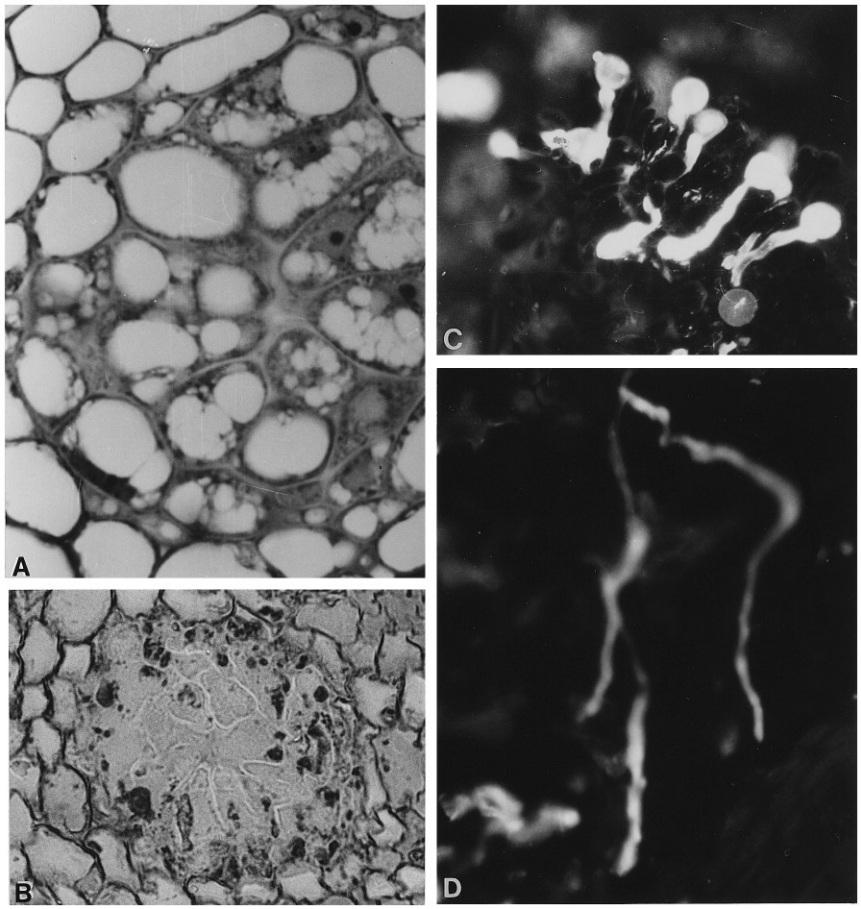

FIG. 4. Cytochemical details of the style, and extent of pollen germination and tube growth. A, Part of transmitting tissue strand (in T.S.) at level 3 (in Fig. 2) stained with toluidine blue magnified to show the presence of sulphated polysaccharides in the intercellular matrix. × 210. B, T.S. of transmitting tissue strand at level 6 (in Fig. 2) stained with Coomassie blue shows absence of proteins in the intercellular matrix. × 170. C and D, Fluorescence photomicrographs of cleared pollinated pistils stained with aniline blue. Pollen grains have germinated on the stigma (C) but pollen tubes have not grown below the upper 2 mm of the style (D). Pollen tubes show thick callose deposition. × 120.

thick callose wall and branching, indicating a lack of suitable conditions for normal tube growth. Variations in the structure of the transmitting tissue of the style and lack of proteins in the extracellular matrix of the transmitting tissue could be the causative factor(s) for the failure of the style to support pollen tube growth. Studies of pollen-pistil interaction in sexual populations of *Commiphora wightii* may be enlightening; however to date, we have been unable to locate any sexual populations. The present study does not determine whether the inability of the style to support pollen tube growth is the cause or result of apomixis in *Commiphora wightii*. More extensive studies on other apomictic species may provide information on the relationship between pollen-pistil interaction and apomixis.

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LITERATURE CITED

Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Annals of Biochemistry* **72**: 248–254.

Chen CG, Mau SL, Clarke AE. 1993. Nucleotide sequence and style-specific expression of a novel proline-rich protein gene from *Nicotiana alata*. *Plant Molecular Biology* **21**: 391–395.

Cheung AY, May B, Kawata EE, Qu G, Wu HM. 1993. Characterization of cDNAs for stylar transmitting tissue-specific proline-rich proteins in tobacco. *The Plant Cell* **3**: 151–160.

Cresti M, Blackmore S, Van Went JL. 1992. *Atlas of sexual reproduction in flowering plants*. Berlin: Springer-Verlag.

Davis GL. 1966. *Systematic embryology of angiosperms*. New York: John Wiley.

Dickinson HG, Moriarty J, Lawson J. 1982. Pollen-pistil interaction in *Lilium longiflorum*: the role of the pistil in controlling pollen tube growth following cross and self-pollinations. *Proceedings of the Royal Society of London Series* B **215**: 45–62.

Feder N, O'Brien TP. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* **55**: 123–142.

Ghosh S, Shivanna KR. 1984. Structure and cytochemistry of the stigma and pollen-pistil interaction in *Zephyranthes*. *Annals of Botany* **53**: 91–105.

Gonzalez MV, Coque M, Herrero M. 1996. Pollen-pistil interaction in kiwifruit (*Actinidia deliciosa*' Actinidiaceae). *American Journal of Botany* **83**: 148–154.

Gupta P, Shivanna KR, Mohan Ram HY. 1996. Apomixis and polyembryony in the guggul plant, *Commiphora wightii*. *Annals of Botany* **78**: 67–72.

Herscovitch JC, Martin ARH. 1989. Pollen-pistil interaction in *Grevillea banksii*. I. The pollen grains, stigma, transmitting tissue and *in vitro* pollinations. *Grana* **28**: 69–84.

Heslop-Harrison J. 1979. Aspects of the structure, cytochemistry and germination of the pollen of rye (*Secale cereale* L.). *Annals of Botany* **44**: 1–47.

Heslop-Harrison J, Heslop-Harrison Y. 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. *Stain Technology* **45**: 115–120.

Heslop-Harrison J, Heslop-Harrison, Y. 1986. Pollen-tube chemotropism: fact or delusion? In: Cresti M, Dallai R, eds. *Biology of reproduction and cell motility in plants and animals*. Siena: University of Siena, 169–174.

Heslop-Harrison J, Knox RB, Heslop-Harrison Y. 1974. Pollen-wall proteins: exine held fractions associated with the incompatibility response in Cruciferae. *Theoretical and Applied Genetics* **44**: 133–137.

Heslop-Harrison Y. 1977. The pollen stigma interaction: pollen tube penetration in *Crocus*. *Annals of Botany* **41**: 913–922.

Heslop-Harrison Y, Heslop Harrison J. 1982. Pollen stigma interaction in the Leguminosae: the secretory system of the style in *Trifolium pratense* L. *Annals of Botany* **50**: 635–645.

Heslop-Harrison Y, Shivanna KR. 1977. The receptive surface of the angiosperm stigma. *Annals of Botany* **41**: 1233–1258.

Heslop-Harrison Y, Heslop-Harrison J, Roger BJ. 1985. The pollen-stigma interaction in the grasses. Pollen-tube guidance and the regulation of tube number in *Zea mays*. *Acta Botanica Neerlandica* **34**: 193–211.

Heslop-Harrison Y, Heslop-Harrison J, Shivanna KR. 1981. Heterostyly in *Primula*. I. Fine structural and cytochemical features of the stigma and style in *Primula vulgaris* Huds. *Protoplasma* **107**: 171–188.

Jensen WA. 1962. *Botanical histochemistry*. London: Freeman WH and Company.

Knox RB. 1984. Pollen-pistil interaction. In: Linskens HF, Heslop-Harrison J, eds. *Encyclopaedia of plant physiology*, *New Series 17*. Berlin: Springer-Verlag, 508–608.

Knox RB, Williams EG, Dumas C. 1986. Pollen, pistil and reproductive function in crop plants. *Plant Breeding Reviews* **4**: 9–79.

Linskens HF, Esser K. 1957. Über eine Spezifische Anafarbung der Pollenschlauche und die Zahl Kalloseпropfen nach selbstung und fremdung. *Naturwissenschaften* **44**: 16.

Lord EM, Heslop-Harrison Y. 1984. Pollen-stigma interaction in Leguminosae: stigma organisation and the breeding system in *Vicia faba* L. *Annals of Botany* **54**: 827–836.

Lord EM, Sanders LC. 1992. Roles for the extracellular matrix in plant development and pollination: a special case of cell movement in plants. *Developmental Biology* **153**: 16–28.

McGuckin WE, McKenzie BF. 1958. An improved periodic acid fuchsin sulphite staining method for evaluation of glycoproteins. *Clinical Cytochemistry* **4**: 476–483.

Mattsson O, Knox RB, Heslop-Harrison J, Heslop-Harrison Y. 1974. Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. *Nature* **247**: 298–302.

Owens SJ. 1989. Stigma, pollen and pollen-stigma interaction in Caesalpinioideae. In: Stirton CH, Zarruchi JL, eds. *Advances in legume biology*. Monographs in Systematic Botany from the Missouri Botanical Garden **29**: 113–126. St. Louis: Missouri Botanical Garden.

Richards AJ. 1990. Studies in *Garcinia*, dioecious tropical forest trees: agamospermy. *Botanical Journal of Linnean Society* **103**: 233–250.

Sanders LC, Lord EM. 1992. A dynamic role for the stylar matrix in pollen tube extension. *International Reviews in Cytology* **140**: 297–318.

Sassen MA. 1974. The stylar transmitting tissue. *Acta Botanica* **23**: 99–108.

Shivanna KR, Johri BM. 1985. *The angiosperm pollen: structure and function*. New Delhi: Wiley Eastern.

Shivanna KR, Rangaswamy NS. 1992. *Pollen biology: a laboratory manual*. Heidelberg: Springer-Verlag.

Shivanna KR, Cresti M, Ciampolini F. 1997. Pollen development and pollen-pistil interaction. In: Shivanna KR, Sawhney VK, eds. *Pollen biotechnology for crop production and improvement*. Cambridge: Cambridge University Press, 15–39.

Tilton VR, Horner HJ Jr. 1980. Stigma, style and obturator of *Ornithogalum caudatum* (Liliaceae) and their function in the reproductive process. *American Journal of Botany* **67**: 1113–1131.

Wang H, Wu H, Cheung AY. 1993. Development and pollination regulated accumulation and glycosylation of a stylar transmitting tissue specific proline-rich protein. *The Plant Cell* **5**: 1639–1650.

Wu HM, Wang H, Cheung AY. 1995. A pollen tube growth stimulatory glycoprotein is deglycosylated by pollen tubes and displays a gradient of glycosylation in the flower transmitting tissue. *Cell* **82**: 393–403.