The Plant Cell, Vol. 2, 39–49, January 1990 © 1990 American Society of Plant Physiologists

Brassica S-Proteins Accumulate in the Intercellular Matrix along the Path of Pollen Tubes in Transgenic Tobacco Pistils

Muthugapatti K. Kandasamy, Kathleen G. Dwyer,[1] Dominic J. Paolillo, Ronald C. Doney,[2] June B. Nasrallah, and Mikhail E. Nasrallah[3]

Section of Plant Biology, Division of Biological Sciences, Cornell University, Ithaca, New York 14853

A tobacco plant transformed with a *Brassica oleracea* SLG-22 gene was analyzed by immunocytochemical methods to determine the localization of the transgene-encoded protein product. Immunolabeling was observed in the pistil along the path followed by pollen tubes after pollination. S-antigen accumulated in the intercellular matrix of the transmitting tissue of the style and its continuation in the basal portion of the stigma and outside a few special cells of the placental epidermis of the ovary. This pattern of S-antigen distribution closely resembles that described for the S-associated glycoproteins of self-incompatible *Nicotiana alata* and differs from its distribution in *B. oleracea*.

INTRODUCTION

In the companion paper, Moore and Nasrallah (1990) report on the transformation of tobacco with SLG-13, the structural gene for the S-locus-specific glycoprotein (SLSG) isolated from a *Brassica oleracea* S_{13} homozygote. As characterized by RNA and protein blot analyses and immunolocalization using the light microscope (LM), the *Brassica* SLG-13 gene was expressed in the stylar transmitting tract of transgenic tobacco. This pattern of expression differed markedly from that observed in *Brassica*, where SLG transcripts were exclusively localized to the papillar cells of the stigma surface (Nasrallah, Yu, and Nasrallah, 1988) and SLSG were shown to accumulate in the walls of these cells (Kandasamy et al., 1989). However, it resembled more closely the well-documented expression of incompatibility-associated molecules in self-incompatible *Nicotiana alata*. The prediction that incompatibility protein factors would be found in the style of *Nicotiana* was initially made by East (1934) and borne out by the electrophoretic detection of such proteins in stylar extracts (Bredemeijer and Blaas, 1981). More recent reports have described in detail the expression of *N. alata* S-associated genes by in situ hybridization (Cornish et al., 1987) and by immunocytochemistry (Anderson et al., 1989).

In this paper, we report on the microscopic immunolocalization of *Brassica* SLSG in tobacco plants transformed with an SLG gene from a *B. oleracea* S_{22} homozygous line. We show at the ultrastructural level that the SLG gene product accumulates in the intercellular matrix of the transmitting tissue of the style and its continuation in the base of the stigma and in the secretory matrix of specific cells of the placental epidermis of the ovary.

RESULTS

Expression of the SLG-22 Gene in Transgenic Tobacco

SLG-22, the SLG gene isolated from a *B. oleracea* S_{22} homozygous line, was isolated as described in Methods. The 11-kb EcoRI fragment carrying the gene included 5.6 kb of sequence upstream of the initiating ATG codon and 3.9 kb of sequence downstream of the termination codon. This fragment was inserted into pBI121, a vector that carries a kanamycin resistance gene and a gene coding for β-glucuronidase (Jefferson, Kavanagh, and Bevan, 1987). The construct was introduced into tobacco by *Agrobacterium*-mediated transformation as described in Methods, and several transgenic plants were generated.

Expression of the SLG-22 gene in transgenic tobacco was monitored with MAb/H8, a monoclonal antibody raised to purified SLSG (Kandasamy et al., 1989). This monoclonal antibody is specific to a protein epitope of SLSG and exhibits cross-reactivity with SLSG from a number of S-allele homozygotes including S_{22} but not with tobacco tissues, as shown in the companion paper (Moore

[1] Current address: Biology Department, University of Scranton, Scranton, PA 18510.

[2] Current address: Biology Department, State University of New York, College at Cortland, Cortland, NY 13045.

[3] To whom correspondence should be addressed.

and Nasrallah, 1990). At the level of protein blot analysis and in six transgenic tobacco plants analyzed, expression of the gene was similar to that described for the SLG-13 gene in the companion paper (Moore and Nasrallah, 1990) and was limited to the pistil. Based on the segregation of kanamycin resistance and the β-glucuronidase gene in its selfed progeny, one of the transgenic plants was deduced to carry the introduced DNA stably integrated at one site in the genome (data not shown). The data presented in the remainder of the paper were derived from the analysis of this stably transformed tobacco plant.

Figure 1 shows the detection on immunoblots of the SLG-22-encoded S-antigen in the styles (Figure 1A) and stigmas (Figure 1B) of transgenic tobacco. On isoelectric focusing gels (Figure 1A), this protein (lane 2) exhibited a charge similar to that of SLSG produced in *Brassica* S_{22} stigmas (lane 1). It also exhibited molecular weight heterogeneity following SDS-PAGE (Figure 1B), as does authentic *Brassica* SLSG. The distribution of S-antigen in the stigma was evaluated by analyzing two separate regions obtained by dissecting stigmas under the stereoscope (see Figure 2A): the upper region of the stigma, which included the papillar epidermis and the underlying secretory zone, and the base of the stigma, which included the transition zone to the style. Figure 1B shows that S-antigen was detected in extracts of the upper region (lane 1) and the base (lane 2) of the stigma. A fraction of S-antigen in the former extract exhibited an aberrantly slower mobility on SDS-PAGE similar to that shown for the S-antigen encoded by the SLG-13 transgene in the companion paper by Moore and Nasrallah (1990).

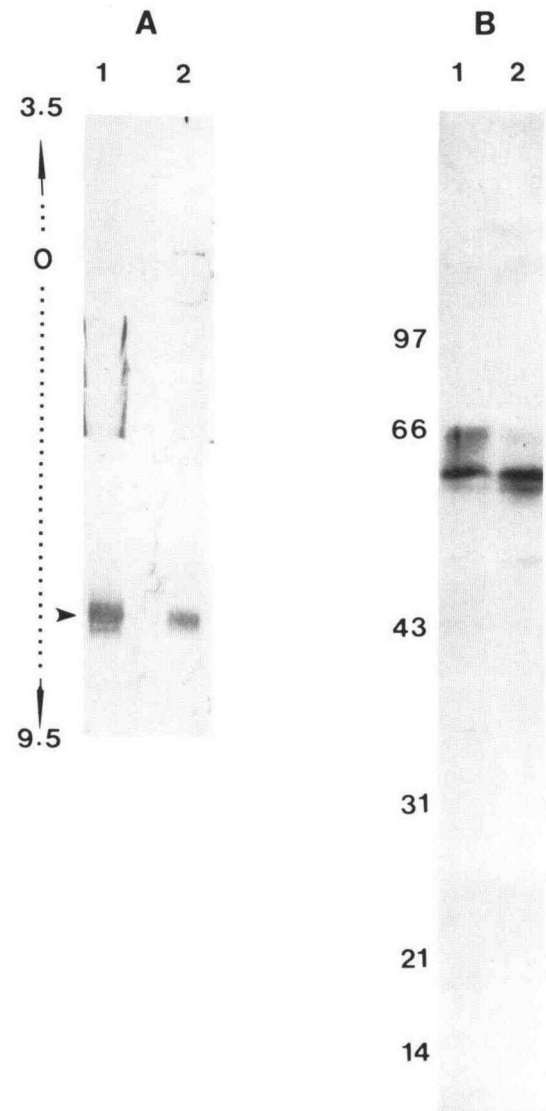


Figure 1. Immunoblot Analysis of S-Antigen.

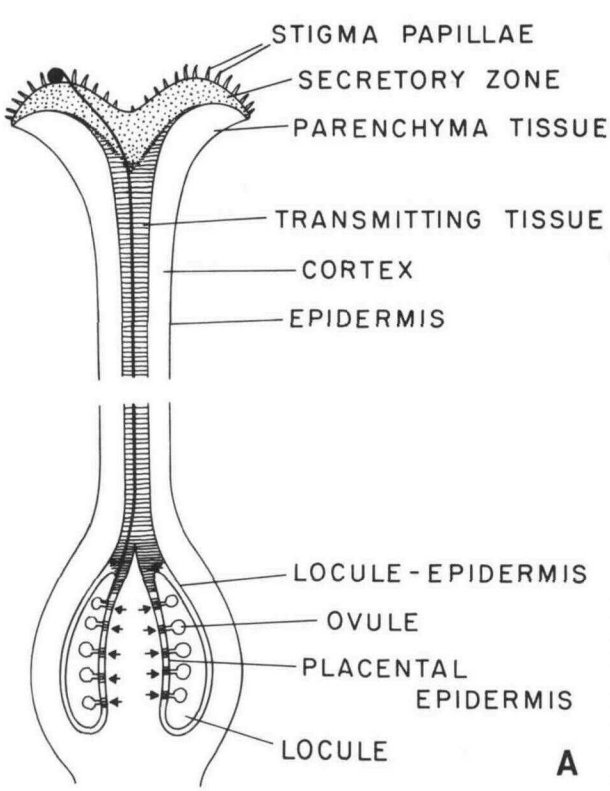
(A) Immunoblot of an isoelectric focusing separation of soluble proteins from *B. oleracea* S_{22} stigmas (lane 1) and transgenic tobacco styles (lane 2). The arrowhead points to the S-antigen band. 50 μg of total protein were loaded in each lane. The pH gradient is indicated to the left.

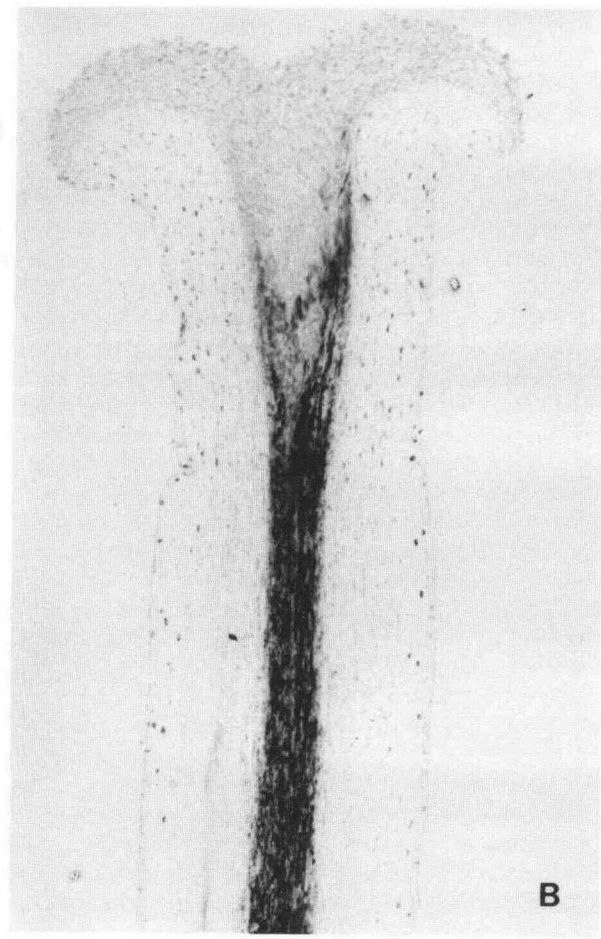
(B) Immunoblot of an SDS-PAGE separation of proteins from transgenic tobacco stigma tissue. The lanes contain 10μg of total protein isolated from the stigmatic region including the papillate epidermis and the secretory zone (lane 1) and from the base of the stigma including the transition region to the style (lane 2). Molecular weight markers are shown to the left.

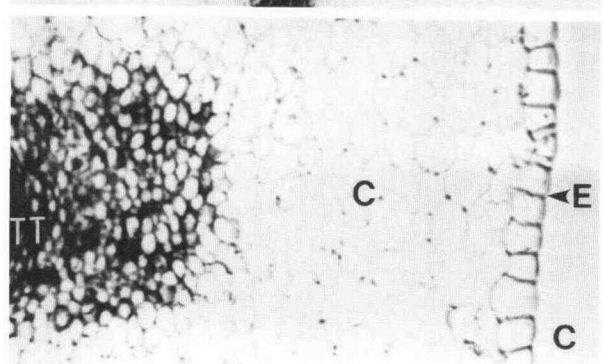
Accumulation of S-Antigen in the Intercellular Matrix of Transmitting Tissue in Transgenic Tobacco Styles

The organization of the stigma (Cresti et al., 1986; Kandasamy and Kristen, 1987) and the style (Bell and Hicks, 1976) of *Nicotiana* is diagrammed in Figure 2A. The bilobed "wet" stigma (Heslop-Harrison and Shivanna, 1977) contains three distinct zones: a papillate epidermis, a subepidermal secretory zone, and a parenchymatous ground tissue. The former two are involved in the production of stigmatic secretion. At maturity, the cells of the secretory zone are separated by large intercellular spaces filled with heterogeneous secretion containing polysaccharides, proteins, and abundant lipids in the form of droplets (Kandasamy and Kristen, 1987). The secretory zone of the stigma converges into the style as a solid core of transmitting tissue, the cells of which are separated by a homogeneous intercellular matrix. This tissue is surrounded by a parenchymatous cortex and the epidermis. After pollination, pollen tubes grow in the style through the intercellular matrix of the transmitting tissue and then in the ovary over the placental epidermis to reach the ovules.

The distribution of the *Brassica* SLG-22 gene product was examined at the light and electron microscopic levels following immunostaining of tissue sections. The overall pattern of positive labeling obtained in transgenic pistils is diagrammatically represented in Figure 2A. With the light







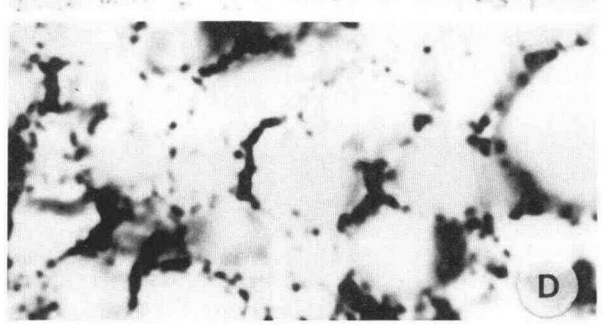


Figure 2. Distribution of SLG-22 Transgene-Encoded S-Antigen in the Pistil of *N. tabacum.*

(A) Mature pistil showing the path of pollen tubes in a compatible pollination and the spatial pattern of expression of the *Brassica* SLG-22 gene. The regions marked by cross-lines labeled positively for *Brassica* S-antigen at the LM and EM levels. This diagram was constructed based on immunocytochemical observations of both longitudinal and transverse sections taken at different levels of the stigma, style, and ovary.

(B) Immunostained longitudinal section through the stigma and style. Magnification ×45.

(C) Immunostained transverse section of a portion of the transmitting tissue (TT), the cortex (C), and the epidermis (E) from the midregion of the style. Staining is only detected in the transmitting tissue. The walls of the epidermis are refractive and are therefore evident in the photograph. Magnification ×320.

(D) Immunostained section through the transmitting tissue, similar to that shown in **(C)**, at higher magnification. The staining of the intercellular matrix is evident. Magnification ×1920.

microscope, a high and uniform level of expression was detected in the transmitting tissue of the style (Figures 2B, 2C, and 2D), with a transitional region of lighter staining at the base of the stigma (Figure 2B). No labeling of the cortical or epidermal cells of the style was evident (Figures 2B and 2C).

The immunolocalization of SLSG obtained by transformation with the SLG-22 gene was compared directly with that obtained with the SLG-13 gene (Moore and Nasrallah, 1990) in longitudinal section using light microscopy. For both genes, the principal site of localization was the transmitting tissue of the style, with the staining reaching highest into the stigma at the periphery of the transmitting tissue. However, staining at the boundary of the transmitting tissue with the cortex could be followed further into the stigma region with SLG-13, and the centrally located epidermis of the stigma was lightly but clearly labeled. No epidermal staining could be proven with SLG-22. Also, in the SLG-13-transformed tissues, the staining of the transmitting tissue was most intense near the base of the stigma and lighter below. In the SLG-22-transformed tissues, the staining was uniform throughout the style to the region of fade-out beneath the stigma.

In the transmitting tissue of the SLG-22 transgenic plant, immunostaining was particularly dense over the intercellular matrix, as observed with the LM (Figure 2D) and the electron microscope (EM), as shown in Figure 3A. Gold particles were concentrated over the matrix along the longitudinal walls, with only sparse labeling over the transverse walls (Figure 3B). It is interesting to note that pollen tubes grow within the intercellular matrix only along the longitudinal walls. Figure 3C shows, in cross-section, pollen tubes present in the intercellular matrix between the longitudinal walls and surrounding a central transmitting cell.

The specificity of the observed labeling was demonstrated by the absence of gold particles in sections from nontransformed tobacco plants (Figure 3D) and in control sections of transgenic pistils in which treatment with primary antibody was omitted from the immunostaining protocol (Figure 3E). In addition, the surrounding layers of cortical parenchyma cells (Figure 3F) and outer epidermal cells of the style showed no bound gold particles.

S-Antigen Levels during Pistil Maturation

Ten different developmental stages from 8 days before anthesis to 1 day after anthesis and pollination were examined at the ultrastructural level. The transmitting tissue from very young pistils at 6 days to 8 days before anthesis did not show labeling with gold particles, as seen in Figure 4A. S-antigen could be detected in the intercellular matrix of the transmitting tissue as early as 5 days before anthesis (Figure 4B). The labeling was weak in the early stages (Figures 4B and 4C). However, there was a significant increase in the amount of labeling during maturation of the pistil (Figures 4D and 4E) as the width of the matrix between the primary walls increased (compare Figures 4B and 4E). Maximal accumulation of S-antigen was observed in the intercellular matrix of open flowers (Figure 4E).

Under the fixation and staining conditions used, the cytoplasm of the transmitting cells themselves exhibited only light labeling. However, gold particles were often observed over the primary walls of these cells near the plasma membrane or on the plasma membrane itself (Figure 4D), an indication of the secretion of S-antigen from the cytoplasm into the intercellular matrix.

Distribution of S-Antigen in the Stigma and Ovary of Transgenic Tobacco

In agreement with the LM observations, electron microscopic examination of sections along the length of the transgenic tobacco pistil showed that labeling of the intercellular matrix was uniform along the length of the style and extended into the basal transition region of the stigma. In the stigma, neither the papillae nor the cells of the secretory zone were labeled at the EM level, as seen in Figures 5A and 5B, respectively. Similarly, there was no labeling of the parenchymatous ground tissue. In serial

Figure 3. Immunogold Localization of *Brassica* S-Antigen in the Stylar Transmitting Tissue of Transgenic *N. tabacum*.

(A) Open flower stage. Transmitting cells from the basal portion of the style. Note the heavy labeling over the intercellular matrix (IM). Magnification ×13,480.

(B) Longitudinal section of transmitting cells at 1 day before anthesis. Gold particles are visible over the intercellular matrix (IM) along the longitudinal cell walls. The transverse wall (TW), which contains many plasmodesmata, shows very poor labeling. Magnification ×12,250.

(C) Transverse section of the transmitting tissue (TT) approximately 1 day after pollination. Pollen tubes (PT) are seen in the intercellular matrix (IM), which is labeled with gold particles. Magnification ×12,070.

(D) Immunocytochemical control showing lack of labeling of the intercellular matrix in a transverse section of stylar transmitting tissue from untransformed *N. tabacum* at the open flower stage. Magnification × 14,200.

(E) Immunocytochemical control in which the sections were treated with secondary antibody without prior labeling with MAb/H8. Transmitting cells from transgenic *N. tabacum* at 1 day before anthesis are shown. Note the absence of gold particles over the intercellular matrix (IM). Magnification ×14,600.

(F) Parenchyma cells from the stylar cortex of transgenic *N. tabacum* showing the absence of immunogold labeling. IS, intercellular space. Magnification × 9600.

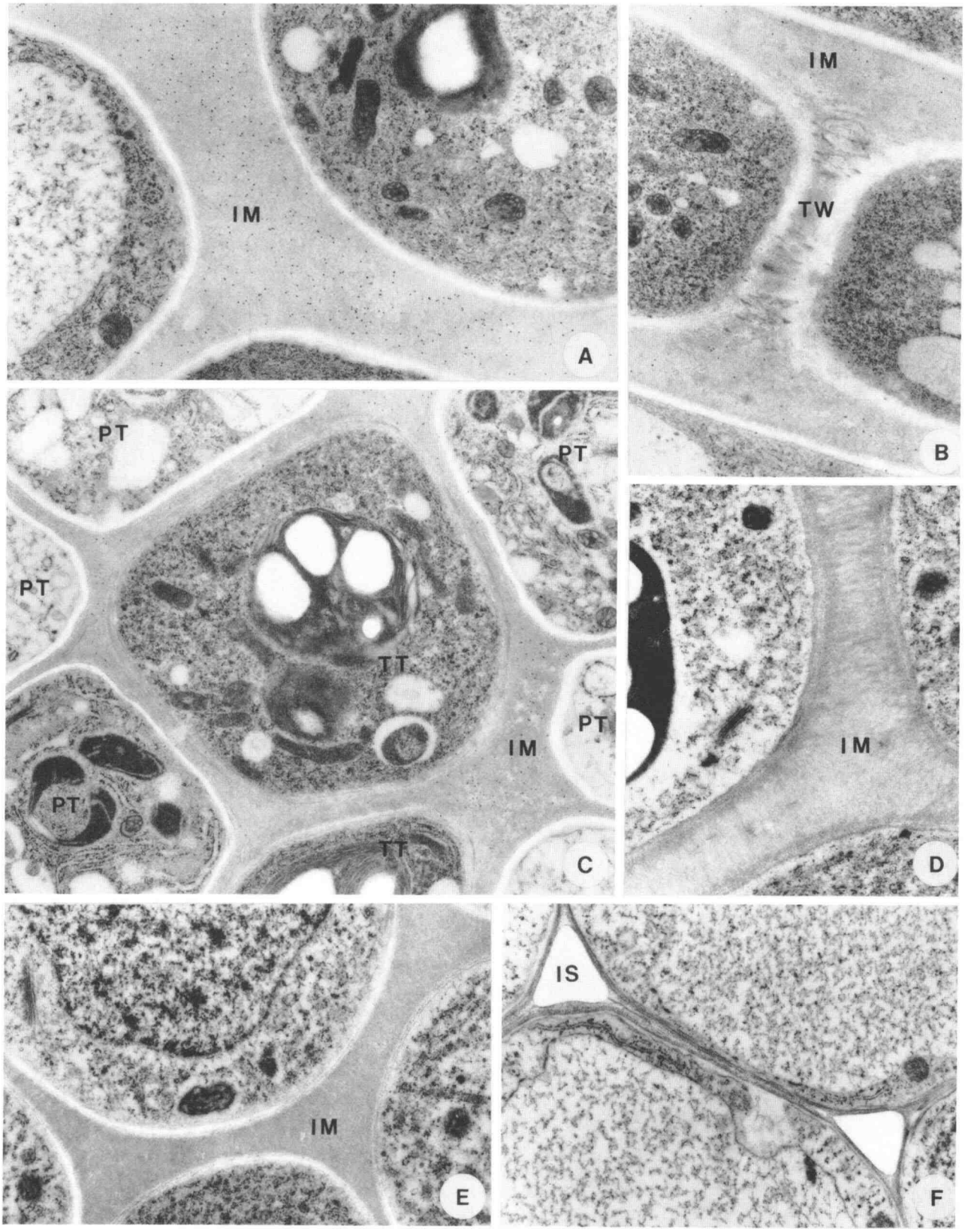


Figure 3.

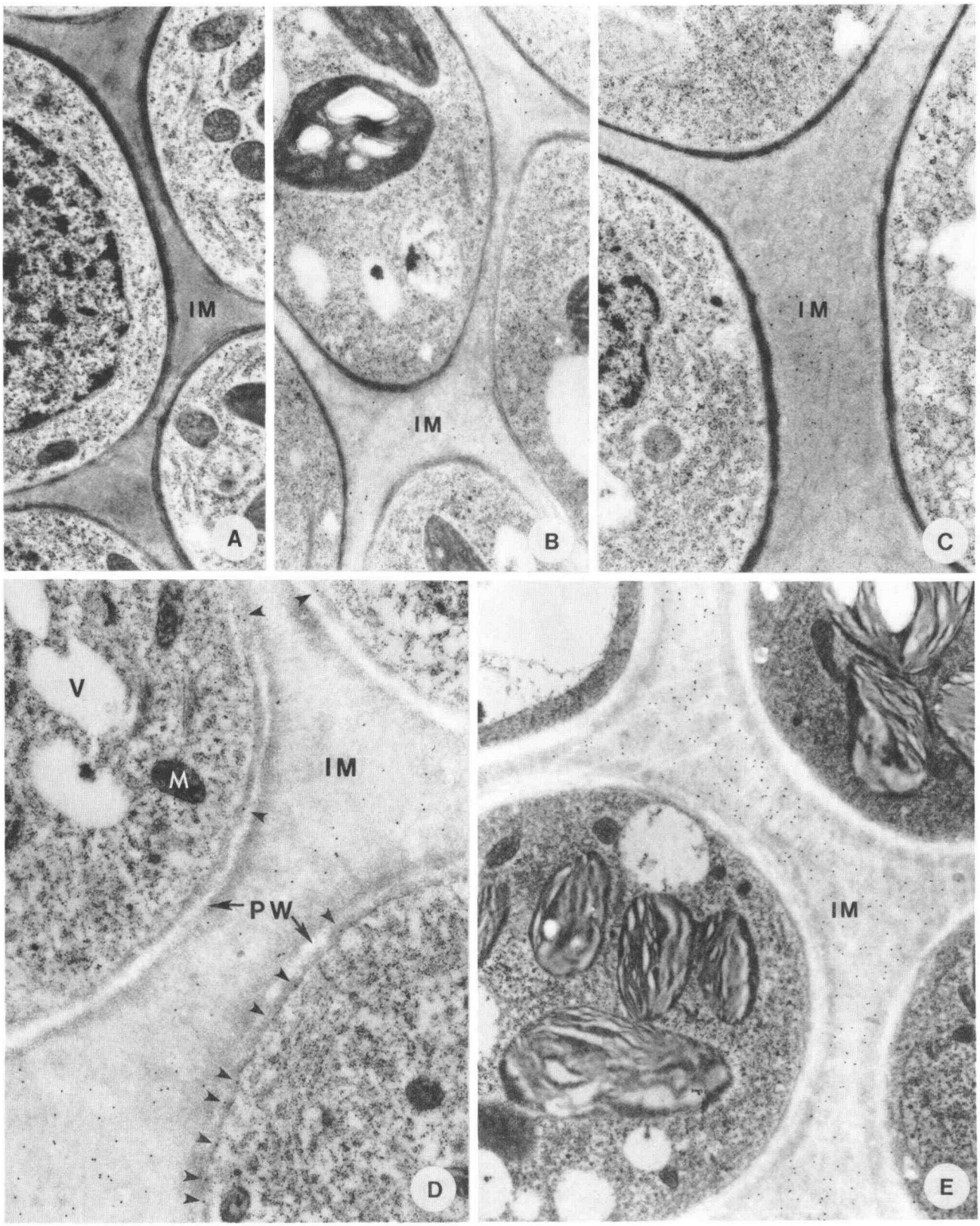


Figure 4. Immunocytochemical Localization of *Brassica* S-Antigen in Transverse Sections through the Stylar Transmitting Tissue of Transgenic *N. tabacum* at Different Developmental Stages.

sections toward the base of the stigma, gold particles were first evident in the intercellular spaces between the secretory cells of the transition region, interspersed with lipid droplets (Figure 5C). Labeling was also detected in a subset of cells of the ovary, where only the placental epidermal cells at the point of attachment of ovules with the placenta showed gold particles. The epidermal cells away from the ovules did not show detectable labeling for S-antigen (Figure 5D). In those epidermal cells near the ovules, gold particles were observed over the secretory matrix surrounding these cells toward the locule (Figure 5E) and toward the adjacent layer of placental cells (Figure 5F). There was no labeling in the ovules or other cells of the ovary.

DISCUSSION

The results of the immunolabeling studies reported in this paper have defined the spatial pattern of expression of the *Brassica* SLG-22 gene in the transgenic tobacco pistil at the light and electron microscope level. Our results are consistent with those obtained, using other techniques, for transgenic tobacco plants transformed with another SLG gene (Moore and Nasrallah, 1990). In particular, the SLG-22 gene was specifically expressed in pistils. Also, the transgenic plants remained self-compatible, and self-pollen tube growth was not significantly different from that of untransformed control plants (data not shown). This result may be ascribed to the apparent lack of activity of the SLG promoter in anther tissue, at least at the level of resolution of the experiments described in the companion paper (Moore and Nasrallah, 1990). Alternatively, *Brassica* SLSG may not be competent to mediate an incompatibility response in tobacco. The latter question becomes especially significant if a low level of expression of the SLG gene in transgenic anther tissue can be demonstrated in experiments currently under way and using methods capable of detecting a few molecules of gene product.

In the pistil, we have observed the highest levels of S-antigen in the transmitting tissue of the style. The earliest immunocytological detection of S-antigen was in young buds at 5 days before anthesis and coincided well with the earliest detection of SLG transcripts at the same bud stage by RNA blot analysis. In addition, and as indicated by the density of bound gold particles, maximal levels of S-antigen were attained in mature buds and flowers, a suggestion that the SLG gene product is stable in the tobacco pistil.

The ability of ultrastructural immunocytochemistry to identify the cellular and subcellular location of the antigen of interest has allowed us to define further the details of SLG gene expression in transgenic tobacco. In the style, S-antigen was detected in the intercellular matrix of the transmitting tissue, where it apparently accumulates. A lower level of labeling was also observed over the intercellular matrix between the secretory cells in the basal transition region of the stigma and over the secretory matrix of the placental epidermal cells adjoining the ovules. The intercellular accumulation of S-antigen indicates that this protein is correctly targeted. It apparently follows a pathway of secretion across the cell membrane, which results in its localization in the papillar cell wall in *Brassica* and in the intercellular matrix of the transmitting tissue in *Nicotiana*.

It is noteworthy that, under the tissue fixation and subsequent immunolabeling conditions used, gold particles were not detected in cells of the papillar epidermis and cells of the adjacent secretory layers of the stigma in the SLG-22-transformed tissues, whereas a light but evident staining of centrally located stigma epidermis was obtained for the SLG-13-transformed tissues. Immunoblot analysis has shown, however, that S-antigen encoded by the SLG-22 transgene is found in a region of the stigma that includes the papillate epidermis and the underlying secretory zone (Figure 1B, lane 1). The failure to detect S-antigen in this region by immunocytochemistry may be related to technical limitations of the method. It is possible that, in the papillar cells and/or the underlying secretory cells of the stigma, the level of antigen is below the requisite cytochemical threshold level or is rapidly secreted as a component of the stigma exudate and lost during tissue processing for cytology.

The slightly different spatial patterns of expression of the SLG-22 and SLG-13 transgenes, particularly in cells of the stigma, may be due to differences in integration sites, in the Ti vectors used, or to inherent differences in promoter sequence between the two genes. Nevertheless,

Figure 4. (continued).

(A) 6 days before anthesis. The narrow intercellular matrix (IM) is not labeled with gold particles. Magnification ×13,150.

(B) 5 days before anthesis. The intercellular matrix (IM) is weakly labeled. Gold particles can be detected starting with this stage of development. Magnification ×15,300.

(C) 3 days before anthesis. Many gold particles are visible over the thickened matrix (IM). Magnification ×16,100.

(D) 1 day before anthesis. Gold particles are present over the intercellular matrix (IM) as well as over the primary walls (PW) near the plasma membrane or on the plasma membrane itself (arrowheads). The cytoplasm shows weak labeling. The mitochondria (M) and the small vacuoles (V) are not labeled. Magnification ×22,385.

(E) Open flower stage. Note the heavy labeling over the intercellular matrix (IM) between the transmitting cells. Magnification ×12,160.

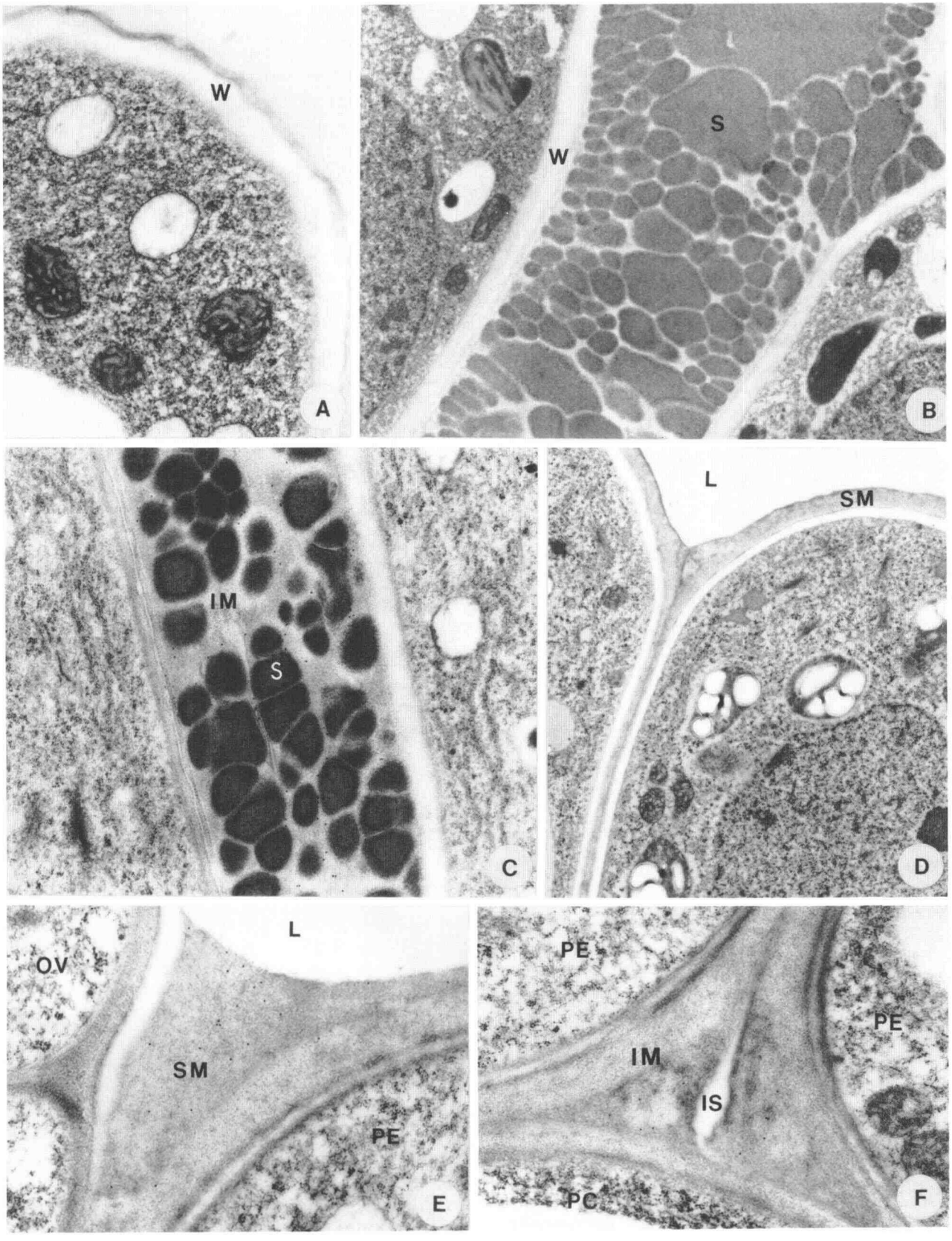


Figure 5. Immunocytochemical Localization of *Brassica* S-Antigen in the Stigma at 1 Day before Anthesis and in the Ovary from Open Flowers of Transgenic *N. tabacum*.

our results allow us to generalize and extend the conclusion made in the companion paper by Moore and Nasrallah (1990) that the expression of *Brassica* SLG genes in transgenic tobacco is more similar to the pattern observed for the *N. alata* S-associated gene (Cornish et al., 1987; Anderson et al., 1989) than to that observed for the same genes in their native *Brassica* context (Nasrallah et al., 1988; Kandasamy et al., 1989). Clearly, SLG gene expression in stylar tissue and in cells of the placental epidermis of the ovary is in sharp contrast with its exclusive expression in the papillar cells of the *Brassica* pistil.

Based on microscopic observations in crucifers and solanaceous plants, there is no obvious structural basis for this difference in expression pattern. The structure of the transmitting tissue does not appear to be drastically different in the two families (Sassen, 1974; Hill and Lord, 1987). The chemical composition of the intercellular matrix is similarly rich in pectin and other polysaccharides (van der Pluijm and Linskens, 1966; Kroh and Munting, 1967), although the apparent absence of proteins in the matrix of crucifers is notable (Hill and Lord, 1987). In addition, and because the intercellular matrix serves as the path for pollen tube growth in both families (de Nettancourt et al., 1973; Hill and Lord, 1987), its function is assumed to be similar to and related to various aspects of pollen tube development. The intercellular matrix of the transmitting tissue has been viewed traditionally as a nutritive medium for the growing pollen tubes. More recently, based on the movement of latex beads through the stylar transmitting tissue of different species, Sanders and Lord (1989) have suggested that the intercellular matrix may also play an active role in the directional movement of pollen tubes.

The similar pattern of expression in tobacco of the *Brassica* SLG genes and the *Nicotiana* S-associated genes implies some similarity in their *cis*-acting regulatory elements and the presence in the tobacco pistil of the appropriate factors required for the activation of the SLG gene. Other plant transformation experiments with tissue-specific genes have shown that the introduced gene is expressed in the same cell types as in the donor plant (Barker, Harada, and Goldberg, 1988). It is possible that the expression of SLG genes in transgenic tobacco represents a cell lineage-specific pattern of expression. Analysis of graft chimeras of *Datura*, a member of the Solanaceae, has shown that the secretory cells of the stigma surface and of the transmitting tissue and cells of the ovarian placental epidermis are all derived from the same epidermal (L1) layer (Satina, 1944). Expression of SLG genes might, thus, reflect the action of specific protein factors found in these cells with common developmental history in *Nicotiana*. Similar protein factors would presumably be limited in their distribution to the papillar cells in *Brassica*.

METHODS

Isolation of the S_{22} SLG Gene and Plant Transformation

An 11-kb EcoRI restriction fragment was identified on genomic DNA blots of *Brassica oleracea* S_{22} homozygotes as containing the SLG gene by hybridization to a gene-specific probe derived from the untranslated 3′ end of SLSG-encoding cDNA (Nasrallah et al., 1988). A subgenomic library enriched in this fragment was constructed in the bacteriophage vector λ GEM11. Positive clones were identified by hybridization to the gene-specific probe, and the insert carrying the SLG gene was inserted into the Ti vector pBI121 (Jefferson et al., 1987). Mobilization into *Agrobacterium tumefaciens* and transformation of *Nicotiana tabacum* were as described in Horsch et al. (1985).

Plant Material

Pistils at various stages of development from 8 days before anthesis to 1 day after anthesis and pollination were obtained from transgenic and nontransformed *N. tabacum* cv Petit Havana plants grown under greenhouse conditions. The different developmental stages were determined based upon the size and age (time before and after anthesis) of the flower buds/flowers.

Tissue Preparation and Immunogold Silver Staining for Light Microscopy

Portions of pistils including the stigma and a part of the style and different regions of the style from a plant transformed with SLG-22 and another plant transformed with SLG-13 (Moore and Nas-

Figure 5. (continued).

(A) A portion of a stigmatic papillar cell. Note the absence of labeling over the cell wall (W) or in the cytoplasm. Magnification ×25,840.
(B) Longitudinal section through the secretory zone of the stigma. The cells are separated by large intercellular spaces filled with secretion. There is no detectable labeling on the cell wall (W) or over the intercellular secretion (S). Magnification ×10,330.
(C) Longitudinal section of secretory cells in the basal transition region of the stigma. Note the positive labeling over the intercellular ground matrix (IM) containing secretory droplets (S). Magnification ×16,800.
(D) Cells of the placental epidermis away from the stalk of the ovule. Gold particles are absent over the secretory matrix (SM) covering the cells toward the locule (L). Magnification ×9250.
(E) Portion of a placental epidermal cell (PE) near the ovule (OV). Gold particles are present over the secretory matrix (SM) covering the cell toward the locule (L). The cells of the ovule are not labeled. Magnification ×27,750.
(F) Intercellular matrix (IM) between the placental epidermal cells (PE) and an adjacent placental cell (PC). Gold particles are seen over the matrix surrounding a narrow intercellular space (IS). Magnification ×21,450.

rallah, 1990) were fixed in a mixture of phosphate-buffered 1% (v/v) glutaraldehyde and 4% (w/v) paraformaldehyde for 2 hr at 20°C, dehydrated in a graded ethanol series, and embedded in paraffin. 10-μm sections mounted on glass slides were processed for immunogold silver staining as previously described (Kandasamy et al., 1989).

Tissue Preparation for Electron Microscopy

Stigmas, styles, and ovaries (dissected into 1-mm to 2-mm pieces) were fixed for 2 hr at 4°C in a mixture of 1% (v/v) glutaraldehyde and 4% (w/v) paraformaldehyde in 50 mM sodium phosphate buffer, pH 7.2, containing 2.5% (w/v) sucrose. The samples were washed several times with phosphate buffer, post-fixed for 1 hr in 1% (w/v) buffered osmium tetroxide at 4°C, washed again in buffer, dehydrated in a graded ethanol series (10% to 100%), and embedded in LR White (Polysciences, Warrington, PA). The resin was changed several times over a 2-day period and then the samples were polymerized at 60°C for 24 hr. Silver-gold transverse and longitudinal sections of different organs of the pistil were obtained with a diamond knife and were mounted on Formvar-coated nickel grids.

Immunogold Labeling for Electron Microscopy

S-antigen was localized on thin sections using an indirect immunogold labeling procedure described previously (Kandasamy et al., 1989). The sections on grids were treated with aqueous sodium metaperiodate and 0.1 N HCl to unmask the antigens (Bendayan and Zollinger, 1983; Craig and Goodchild, 1984). After washing on drops of distilled water, the pretreated sections were incubated in TBST-BSA [50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% (v/v) Tween 20, 0.5% (w/v) BSA] for 15 min, labeled for 1 hr with a 1:50 dilution of the primary antibody MAb/H8 in TBST-BSA, and washed in TBST-BSA. The sections were then treated for 1 hr with a 1:50 dilution of 10 nm colloidal gold-conjugated goat-antimouse IgG secondary antibody. After immunolabeling, the sections were washed with TBST-BSA and then with distilled water and stained with 5% (w/v)aqueous uranyl acetate, followed by alkaline lead citrate for 5 min each.The grids containing labeled sections were examined, and electron micrographs were taken with a Philips EM 300 electron microscope (Philips Netherland, Eindhoven, The Netherlands).

Immunocytochemical Controls

The specificity of labeling was tested by (1) labeling sections from pistils of an untransformed tobacco plant; (2) applying secondary antibodies to sections of transgenic tobacco pistils without prior exposure to the primary antibody, MAb/H8.

ACKNOWLEDGMENTS

This work was supported by grants from the U.S. Department of Energy (DE-FG02-88ER 13909) to M.E.N., and from the U.S. Department of Agriculture (85-CRCR-1-1600) to J.B.N.; (87-CRCR-1-2401) to D.J.P. R.C.D. was supported by a grant from CIBA-GEIGY to M.E.N. and J.B.N., and K.G.D. was supported by a McKnight Foundation post-doctoral fellowship. We thank Drs. Mary Thorsness and Beth Lalonde for reading and improving the manuscript and for helpful discussions during the course of this work.

Received August 4, 1989; revised November 17, 1989.

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