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ULTRASTRUCTURAL STUDIES ON STYLES AND POLLEN TUBES OF ZEA MAYS L. GENERAL SURVEY ON POLLEN TUBE GROWTH IN VIVO

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SUMMARY

Pollen tubes of maize enter the silk intercellularly via multicellular hairs located on two opposite sides of the silk or epidermis cells of the silk proper. The silk contains two vascular bundles. A strand of "transmitting tissue" is associated with each bundle, separated from xylem elements by one parenchyma layer. The pollen tubes grow intercellularly towards the "transmitting tissue" and then between the cells of this tissue down the silk. The diameter of the pollen tubes ranges between 6–13 μm but increases to 26–55 μm at the enlarged section of the tube.

1. INTRODUCTION

Most of the electron microscopic studies concerning pollen tube growth in vivo were done with plant species the flowers of which contain either an open style with a canal or a solid style with one strand of transmitting tissue through which the pollen tubes grow. As discovered by light microscopic studies, pollen tubes of *Zea mays* grow in a special tissue associated with the two vascular bundles of the silk (style). This tissue is interpreted as a bundle sheath (MILLER 1919; KIESSELBACH 1949). While growing through the silk a striking local enlargement of the pollen tube at or in the neighbourhood of the tip was observed (MILLER 1919; KIESSELBACH 1949). *Zea mays* has silks ranging from less than 1 to over 30 cm in length. The pollen tubes, developed from 3-nucleate pollen grains with one germ pore, require, on the average, 24 hrs to reach the ovary (MILLER 1919). During this short period of time, large quantities of tube wall carbohydrates must be synthesized. These features which, in many respect, are uniquely associated with maize stimulated these ultrastructural studies. This report presents a preliminary morphological description of the behavior of pollen tubes after pollen has germinated on the receptive surface of the silk.

2. MATERIALS AND METHODS

Zea mays L. plants were grown under greenhouse conditions and young ears were bagged before silk emergence. After the silks had emerged 3–4 cm they were directly pollinated or cut back to 1 cm and 16 hours later pollinated by freshly-collected pollen. For transmission electron microscopy (TEM) 2 mm pieces of

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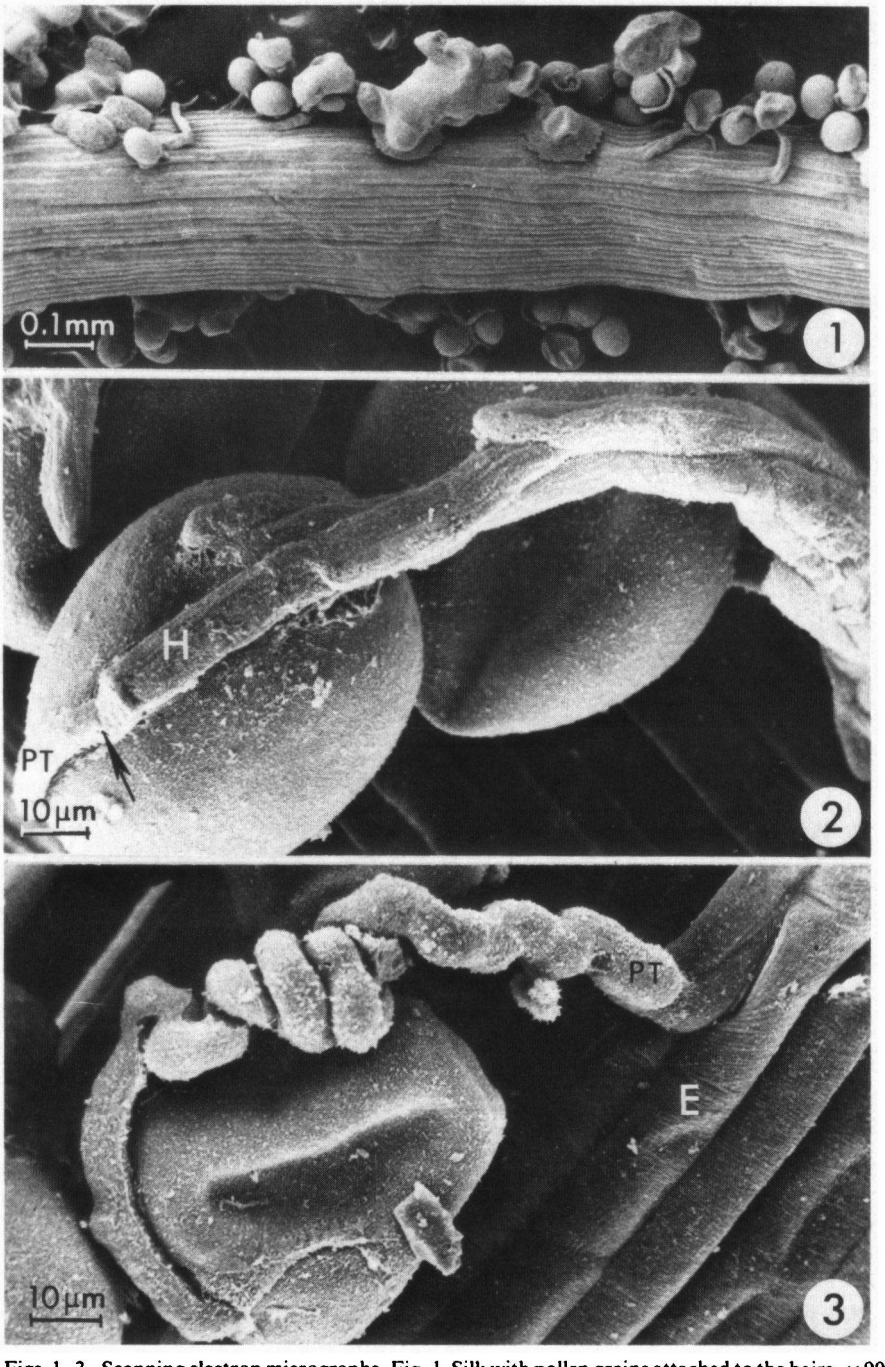
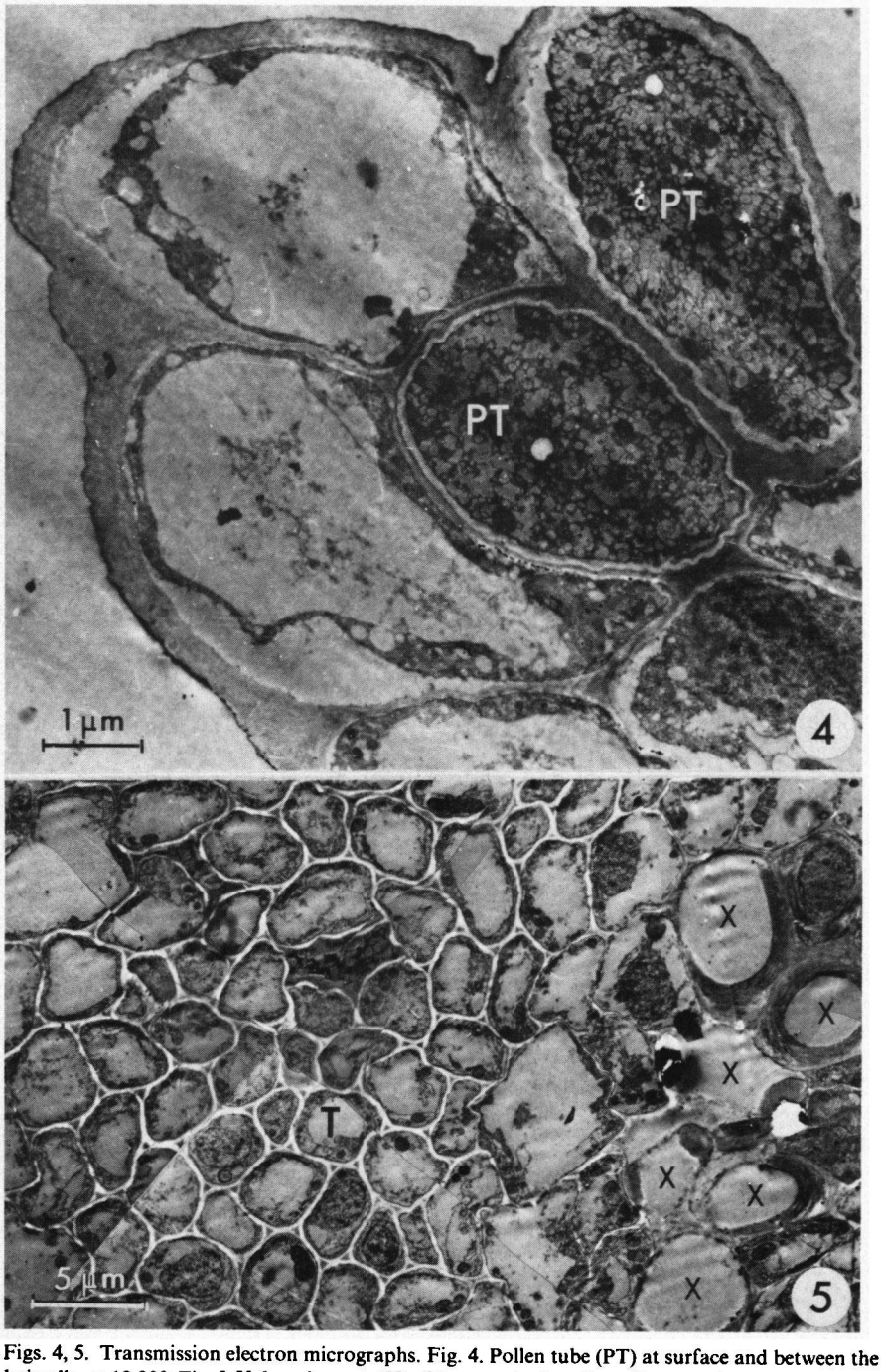


Figs. 1–3. Scanning electron micrographs. Fig. 1. Silk with pollen grains attached to the hairs. ×90. Fig. 2. Pollen tube (PT) attached (arrow) to the top cell of a hair (H). ×820. Fig. 3. Pollen tube (PT) penetrating a silk between the epidermal cells (E). ×930.



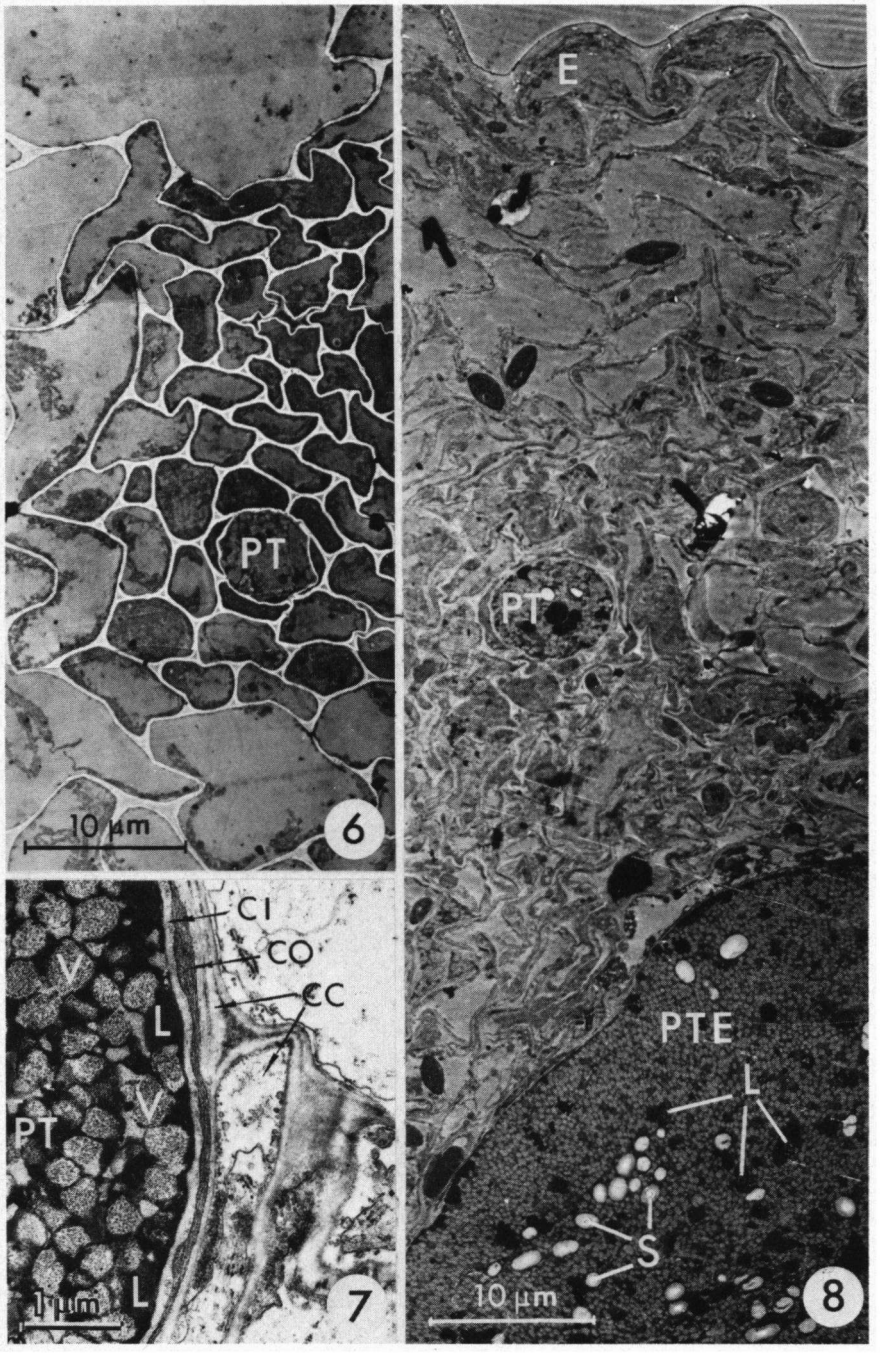
Figs. 4, 5. Transmission electron micrographs. Fig. 4. Pollen tube (PT) at surface and between the haircells. × 12,800. Fig. 5. Xylem elements (X) of the vascular bundle and "transmitting tissue" (T) in silk. × 3,000.

the upper half of the silk 3 to 6 hrs after pollination were fixed in 2% $KMnO_4/H_2O$ for 2 hrs or in 6.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 hrs. After washing, the glutaraldehyde-fixed specimens were postfixed in 2% OsO_4/H_2O for 2 hrs. The specimens were dehydrated in a graded ethanol-propylene oxide series and embedded in Epon. To obtain a preliminary indication of pollen tube growth in vivo, the embedded pollinated silks were cross-sectioned. The sections from the glutaraldehyde-OsO_4 fixed material were stained with uranyl acetate (25 min) and/or lead citrate (12 min), the sections from $KMnO_4$-fixed material only with lead citrate. For scanning electron microscopy (SEM), the pollinated silks were fixed in glutaraldehyde-OsO_4 and dehydrated as described for the TEM prior to critical-point drying and then coated with gold.

3. RESULTS AND DISCUSSION

After pollination, the pollen grains are attached to the multicellular hairs which cover the silks locally on two opposite sides (*fig. 1*). After germination the pollen tubes may enter a hair at different positions. Often the tubes grow first along the surface of the hair cells (*fig. 2*) before entering the hair. Pollen tubes are also able to enter the silk directly (*fig. 3*). The tubes always penetrate a silk hair between the hair cells or the silk proper between the epidermal cells. In the hair, the tubes grow intercellularly to the silk (*fig. 4*). The silk includes two vascular bundles, each containing 5 to 6 xylem elements. Phloem elements could not be identified with certainty on the cross sections. The xylem elements of each bundle are separated by one parenchyma layer from a tissue the cells of which have a smaller diameter than the neighbouring parenchyma cells and a conspicuous cell wall (*fig. 5*). This tissue may be interpreted as a "transmitting tissue" rather than as a bundle sheath, because the pollen tubes, after having penetrated the silk, grow intercellularly towards this tissue and then further down the silk between the cells of this tissue (*fig. 6*). Forcing their way through the parenchyma and "transmitting tissue", the pollen tubes compress, in part, the surrounding cells (*figs. 6–8*). According to MILLER (1919), the distal section of the tube collapses and the compressed cells should regain their normal shape and position. The diameter of a pollen tube in the silk can vary between 6 μm and 13 μm. However, some pollen tube sections with diameters from 26 μm up to 55 μm were also found (*fig. 8*). These obviously correspond to the enlargement described by MILLER (1919) and KIESSELBACH (1949). The enlarged section is assumed to contain the two sperm cells and the vegetative nucleus (KIESSELBACH 1949). In the pollen tubes, membrane-bound vesicles with an electron-dense content predominated over

Figs. 6–8. Transmission electron micrographs. Fig. 6. Pollen tube (PT) surrounded by the compressed cells of the 'transmitting tissue'. × 2,100. Fig. 7. Pollen tube (PT) surrounded by the compressed cells (CC). vesicles (V), lipid vacuoles (L), outer cell wall (CO), inner cell wall (CI). × 12,900. Fig. 8. Cross section of the pollinated silk with two pollen tubes. epidermis (E), normal pollen tube (PT), enlarged pollen tube (PTE), starch grains (S), lipid vacuoles (L). × 2,100. ▶


PT
10 µm
6
E
PT
CI
CO
CC
V
L
V
PT
1 µm
L
7
PTE
L
S
10 µm
8

lipid vacuoles and starch grains (*figs. 7, 8*). Also, the pollen grains of maize contain numerous vesicles (Kroh et al., unpublished results). In this respect, maize pollen resembles the pollen of barley (CASS & PETEYA 1979) and *Impatiens* (VAN WENT 1974). The pollen tube wall of maize consists of two layers, an outer electron-dense fibrillar layer and an inner less electron-dense layer (*fig. 7*).

Subsequent work is being directed towards a detailed study of the ultrastructure of the vascular bundle, the transmitting tissue and the pollen tube. Special attention is being paid to the enlarged section of the pollen tube, its content, the texture of its cell wall and the fate of the distal part of the pollen tube as well as of the cells compressed by the growing tube.

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