Pollen tube growth through nucellus into embryo sac of *Spinacia*— an ultrastructural investigation

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Abstract

The micropylar parts of nucellus and embryo sac were studied in relation to pollen tube growth and its entrance into the embryo sac. The initially homogeneous walls of the cells of the conductive nucellar tissue disintegrate at the middle lamellae region. Pollen tubes pierce the nucellar cuticle and continue their growth into the nucellus intercellularly. Subsequently they can follow various pathways to reach the FA of the degenerated synergid. The penetration into this synergid, and the discharge of the tube contents are described and discussed.

INTRODUCTION

The pollen tubes initially grow in the stigma and style intercellularly but subsequently they follow various pathways (Wilms, 1980). They come out in the space between the carpel and the outer integument and reach the massive nucellus through the micropyle. What happens next is not altogether clear. According to Ramanna and Mutsaerts (1971) the pollen tubes can grow like fungus mycelium and produce haustoria-like structures which penetrate the nucellus and the integuments. Wilms (1974) confirms that of the many tubes which enter the micropyle only a few make their way through the nucellus to the embryo sac, but penetration of the integuments is doubtful. In this article the ultrastructure of the micropylar parts of nucellus and embryo sac are described in relation to the pollen tube growth, and its entrance and discharge into the embryo sac.

MATERIAL AND METHODS

Dissected ovules of *Spinacia oleracea* L., cv. Prévital were fixed in glutaraldehyde and osmium tetroxide at various stages before and after pollination, and embedded in Epon (for detail see Wilms, van Aelst,

1978). Observations were made on a Philips EM 301 at 60 kV. The rate of pollen tube growth was estimated with UV-fluorescence microscopy (Wilms, 1974).

RESULTS

The nucellar tissue between the embryo sac and the micropyle constitute the conductive tissue (Fig. 1) and consists of two cell types. At the micropylar end the micropylar cells have slightly thickened walls (Figs 2, 3), whereas near the embryo sac the cells are short, have thick walls and are designated as transmitting cells (Fig. 5). The boundary between the two cell types is not sharp.

The micropylar cells have initially homogeneous walls but at maturity the region of the middle lamellae as well as the outer wall beneath the cuticle bordering the nucellus disintegrate. The cuticle is affected too (Fig. 2). The cells of the transmitting tissue become loosely organized. Their middle lamellae appear disintegrated, and some electron-dense granules accumulate in the intercellular spaces. Around the conductive tissue the intercellular spaces are small and electron-dense (Figs 4, 5).

Pollen tubes reach the micropyle within 3-5 hr, depending upon the place of contact of the pollen grain with the long stigmas. They can pierce through the cuticle of the nucellar tissue at weak places and grow through the disorganized part of the cell wall (Fig. 2). Subsequently the tubes follow the middle lamellae to the centre of the tissue and continue their growth intercellularly (Fig. 3). When the tubes reach the transmitting cells with their thicker cell walls the tube growth can change. They force their way through the cell wall (Fig. 5), even inside the cell wall, or between the wall and the plasma membrane (Fig. 4). The protoplasm of these penetrated transmitting cells is not affected by the growth of the pollen tubes. Independent of its passage through the transmitting nucellar tissue, the pollen tube reaches the FA of the degenerated synergid. It grows for a short while inside the cytoplasm, and discharges its content by a terminal pore. This content is seen mainly in the extension of the pollen tube and is not mixed completely with the cytoplasm of the degenerated synergid. The vegetative nucleus and the sperm cells are seen close together and frequently in the chalazal part of the degenerated synergid, where they can fuse easily with the egg and the central cells.

DISCUSSION

Pollen tube growth in stigmas and styles have been reported in various ways (Vasil, 1974) but its penetration in the nucellus has not

Fig. 1. Longitudinal section of micropylar part of mature ovule with parts of nucellus and embryo sac

Figs 2-4. Transverse sections of nucellus with pollen tubes as indicated in Fig. 1

Fig. 2. Pollen tube penetration in micropylar cells; cuticle is partly dissolved (arrow). Fig. 3. Intercellular pollen tube growth; conductive tissue is bordered by cells with small electron-dense intercellular spaces (arrow). Fig. 4. Pollen tubes grow intercellularly and between cell wall and plasma membrane; thin-walled cells with small inter-cellular spaces (arrow) border transmitting cells.

CC — central cell, E — egg cell, II — inner integument, IS — intercellular space, OI — outer integument, PT — pollen tube, Sy — synergid.

Fig. 5. Transverse section of nucellar transmitting tissue as indicated in Fig. 1; pollen tube grows through inner part of thick cell walls

Fig. 6. Discharge of pollen tube content in degenerated synergid; pollen tube penetrates filiform apparatus and develops a terminal pore inside the synergid
CC — central cell, CW — cell wall, DS — degenerating synergid, E — egg cell, IS — intercellular space, PT — pollen tube, S — starch grain, V — vacuole.

Point raster indicates the protoplasm of nucellus cells.

been frequently studied, especially in less crassinucellate ovules. Jensen (1969) reports intercellular growth of pollen tubes through a multi-layered nucellus. In spinach the pollen tubes grow in a similar way as they do in the styles i.e., mainly intercellularly (Wilms, 1980). The relatively large intercellular spaces of the conductive nucellar tissue are occupied by pollen tubes. When the cell walls become thicker and the intercellular spaces appear irregular the pollen tubes also pierce through them before reaching the FA of the degenerated synergid. Probably the low molarity of the degenerated cytoplasm of synergid causes its bursting so that its contents, the cytoplasm, two sperm cells and the vegetative nucleus, are discharged into the chalazal part of the synergid. The cell membrane in that portion of the synergid collapses and the pollen tube cytoplasm comes in contact with it and is pressed partly in between the plasma membranes of the egg and central cells.

REFERENCES

- Jensen W. A., 1969. Cotton embryogenesis: pollen tube development in the nucellus. *Can. J. Bot.* 47: 383-385.
- Ramanna M. S., Mutsaerts M. C. A., 1971. Unusual behaviour of growing pollen tubes in the style and ovules of *Spinacia oleracea* L.. *Euphytica* 20: 33-47.
- Vasil I. K., 1974. The histology and physiology of pollen germination and pollen tube growth on the stigma and in the style. In: *Fertilization in Higher Plants* (H. F. Linskens, ed.), North Holland Publ. Co., Amsterdam, pp. 105-118.
- Wilms H. J., 1974. Branching of pollen tubes in spinach. In: *Fertilization in Higher Plants* (H. F. Linskens, ed.), North Holland Publ. Co., Amsterdam, pp. 155-160.
- Wilms H. J., Aelst A. C. Van, 1978. Fertilization in spinach: The pathway of the pollen tubes in the style. *Bull. Soc. Bot. Fr.* 126: 243-247.
- Wilms H. J., 1980. Ultrastructure of the stigma and style of spinach in relation to pollen germination and pollen tube growth. *Acta Bot. Neerl.* 29: 33-47.