ULTRASTRUCTURAL ASPECTS OF THE SELF-INCOMPATIBILITY MECHANISM IN LYCOPERSICUM PERUVIANUM MILL.

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SUMMARY

The experimental results obtained show that the tip of the incompatible pollen tube bursts open after the outer-wall has considerably expanded in the intercellular spaces of the conducting tissue and the inner-wall has disappeared and numerous particles have accumulated in the tube cytoplasm. These particles, which measure approximately 0.2 µm in diameter and give a weak reaction to the test of Thiéry, differ in many respects from the vesicles normally present in compatible pollen tubes growing through the style; they appear to resemble, in some cases, the spheres which are discharged by the compatible pollen tubes after they have reached the embryo-sac.

It is considered that these observations support the current belief that the tube wall is the site of action for the incompatibility proteins and suggest that self-incompatibility is not a passive process resulting from lack of growth stimulation but an active event which leads to the destruction of the incompatible pollen tubes. The degradation mechanism involved appears similar to the one which enables the compatible pollen tube to release its contents in the degenerated synergid and presents some analogies with the lytic process taking place in virus-infected cells.

The general hypothesis is presented that the particles observed in the cytoplasm of self-incompatible pollen tubes consist of a mixture of incompatibility proteins and of basic constituents of the tube wall.

INTRODUCTION

Self-incompatibility in homomorphic gametophytic systems involves, for many plant species, an inhibition of pollen tube growth which takes place when the S-allele in the haploid pollen is identical to one of the two S-alleles present in the diploid style.

Whereas many of the genetical and biochemical features which characterize the self-incompatibility phenomenon are known (Lewis, 1965; Linskens, 1965; Lundquist, 1965; Lewis, Burrage & Walls, 1966; Pandey, 1967, 1970; Nasrallah, Barber & Wallace, 1969; de Nettancourt, Ecochard, Perquin, Van Der Drift & Westerhof, 1971), only a little information is available on the rejection process itself and on the ultrastructural changes which accompany the inhibition of pollen tube growth. Lewis (1965) has suggested that the S-protein complex acts as a genic regulator to
induce either the synthesis of an inhibitor or to repress the synthesis of an auxin of pollen tube growth. Van der Pluijm & Linskens (1966) found that the incompatibility reaction in Petunia hybrida is accompanied by a thickening of the pollen tube wall and the degeneration of the cytoplasm in the tube and endorsed, on the basis of these observations and of a number of biochemical and genetical considerations, the hypothesis of Mäkinen & Lewis (1962) that the incompatibility protein has its place of action on the surface of the pollen tube and not outside in the style. This conclusion has been implicitly supported by Knox, Heslop-Harrison & Reed (1970) and Knox & Heslop-Harrison (1971) who consider that some of the wall-associated proteins detected in mature pollen may be concerned in incompatibility reactions.

In an attempt to gain further information on the mechanism of pollen tube rejection after incompatible pollinations, electron-microscopic observations were made of self- and cross-pollinated pistils of the wild tomato species Lycopersicum peruvianum Mill. This species, characterized by a homomorphic pollen tube-style system of gametophytic monofactorial self-incompatibility (Lamm, 1950; McGuire & Rick, 1954; Gunther, Herrmann & Hoffman, 1968; de Nettancourt et al. 1971), was selected as test material because of the small size of its styles which greatly facilitates fixation and embedding procedures.

MATERIALS AND METHODS

Styles and ovules of S₁S₁, S₂S₂ genotypes, harvested at different time-intervals (16, 24 and 72 h) after pollination with compatible (S₁ and S₂) or incompatible (S₁ and S₂) pollen, were fixed in 5 % glutaraldehyde buffered by 0.065 M Sorensen's phosphate buffer, pH 6.9, for 90 min at room temperature, and postfixed with buffered 1 % osmium tetroxide for 3 h. The tissue was dehydrated through a graded ethanol series and embedded in an Epon-Araldite mixture. Sections were cut with glass knives on an LKB ultramicrotome, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and observed with a Zeiss EM 9 A electron microscope. Some of the sections were tested for polysaccharides using Thiéry's periodic acid-thiocarbohydrazide-silver proteinate technique (Thiéry, 1967). The sectioned material was kept in the TCH/acetic acid solution for 20–24 h; control sections were processed similarly but without any treatment with TCH.

The relationship between time intervals and the localization in the style of pollen-tube apices had been determined previously, for both compatible and incompatible pollen tubes, by means of fluorescence microscopy (de Nettancourt & Ecochard, 1968). The spermatid nuclei in the pollen tubes were stained with basic fuchsin and observed under an ordinary light microscope. Callose deposition in compatible and incompatible pollen tubes was detected by means of the fluorescence technique described by Martin (1958). This method had to be used because callose, with its β-1:3 linked glucan structure and the absence of adjacent hydroxyl groups, cannot be expected to give a positive reaction with the periodate-silver proteinate reagent used in Thiéry's test.

RESULTS

Although no special analysis of the stylar tissue has been made, it was possible to note that the cells composing the conducting tissue have a circular cross-section and are completely separated from one another (Fig. 1). The intercellular spaces are filled by an unknown substance of fairly high electron density which seems to be fairly homogeneous.
Compatible pollen tubes

The sections of the compatible pollen tubes clearly revealed the cylindrical appearance of the apical region (Fig. 2) and the flattening and infolding of the remaining portion of the tube (Fig. 3). Regardless of the time interval between pollination and fixation, the wall of the growing tube is distinctly bipartite with an outer layer consisting of loose fibrils and an inner one which is homogeneous and less electron dense (Figs. 2-4). In the apical region, the tube cytoplasm contains inclusions of irregular sizes and variable shapes which, on the basis of their electron-density, often appear to contain the same material as the inner tube wall (Fig. 4). The aniline blue-fluorescence technique showed that the apical area always contains callose.

When the compatible pollen tube enters the embryo sac, the wall of the tube apparently degenerates and a large number of particles are liberated, within the ovule, in the degenerated synergid where the pollen tube discharges its spermatic nuclei. These particles react weakly to Thiéry’s test and are generally spherical, with a diameter of a few hundred nanometres. They have a granular structure and sometimes appear to be surrounded by a less electron-dense outer shell (Fig. 5).

Incompatible pollen tubes

As in the case of the compatible pollen tubes, the cross-section of the incompatible tube is round in the apical area and infolded in its remaining portion. During the first few hours which follow germination and growth through the first millimetres of the stylar tissue, the incompatible tubes maintain the normal appearance of compatible tubes and clearly display the bipartite tube wall described above (Fig. 6). Later on, when the tip of pollen-tubes has grown through the first third of the style length, the inner wall becomes thinner and numerous particles, about 0.2 μm in diameter and often polyhedral in shape, accumulate in the tube cytoplasm (Fig. 7). These particles, formed by a less electron-dense outer shell and a denser granular core, are strikingly different from the inclusions observed in the compatible tubes. Their general appearance suggests some similarity with the spheres discharged in the embryo sac by compatible pollen tubes. However, in the incompatible pollen tubes, the particles are far more regular in size and shape and clearly present a bipartite structure which was not always distinguishable in the particles located in the micropylar region of the ovules. Thiéry’s test indicated that the polysaccharides detectable by this technique are present in the outer but not in the inner wall of the pollen tube and that the particles are only slightly stained (Fig. 8). The Feulgen test revealed the presence of the 2 spermatic nuclei but failed to detect any significant amount of DNA at the tip of tubes which had accumulated a large number of particles. Observations by means of the fluorescence technique showed that callose, which did not react to Thiéry’s test, outlines practically the whole length of the pollen tube but is absent at the tip of the apical area.

When the cytoplasm in the tube apex is completely loaded with particles, the inner wall of the tube disappears and the outer wall becomes thicker, giving a swollen appearance to the tip of the tube. At this stage, the incompatible tube bursts open
(Fig. 9) and releases the particles in the intercellular spaces of the stylar conducting tissue which is obviously filled with a substance of sufficient fluidity to allow free dispersion of the tube contents. Thiery's test showed that the particles, after their liberation from the tube, do not contain any appreciable amount of the polysaccharides which are detectable by this technique (Fig. 10).

DISCUSSION

Compatible pollen tubes

With regard to the ultrastructure of compatible pollen tubes growing in vivo, our observations are in good agreement with the description made by van der Pluijm & Linskens (1966) on Petunia hybrida and confirm the bipartite structure of the tube wall and the growth of the pollen tube through the intercellular spaces of the stylar tissue. The vesicles observed at the tip of compatible pollen tubes resemble very much the cytoplasmic inclusions described by Sassen (1964), Larson (1965), Rosen (1968) and Van Der Woude, Morré & Bracker (1970) in pollen germinated in vitro and considered by these authors to play an active role in cell wall biogenesis. It is apparent from the present study that some of these vesicles have the same composition as the inner wall of the tube and that their involvement in the formation of the wall is probably restricted to the inner layer only. This conclusion is substantiated by the observation that the outer wall continues to expand in incompatible pollen tubes where the vesicles are no longer produced.

The finding that the compatible pollen tube breaks down in the micropylar region of the embryo sac and releases a large mass of granular particles in the degenerated synergid had been previously reported in Gossypium by Jensen & Fisher (1968), in Zea by Diboll (1968) and in Linum by Vazart (1971). The fact that this phenomenon occurs in genera so widely unrelated as Gossypium, Linum, Zea and Lycopersicum certainly suggests that the mechanism is common among angiosperms. It would be interesting, in this connexion, to know what factors initiate the opening of the tube and how the spherical particles originate. The observations of Jensen & Fisher (1968) and those of Vazart (1971) certainly convey the impression that the synergid degenerates upon a signal from the pollen tube which, in turn, appears to receive, at the time of its passage through the degenerated cell, the necessary information for releasing its content in the embryo sac. One is, in other words, dealing with a system of recognition and of induction which presents some analogy with the self-incompatibility reaction. Hence, the fact that in both cases complex identification processes lead to lysis of the pollen tube and to the formation of numerous particles may not be purely casual.

Incompatible pollen tubes

The present study demonstrates beyond doubt that incompatible pollen tubes are not only inhibited in their growth through the style but are destroyed by a precise degradation process which leads to the disappearance of the inner wall and the lysis of the tube. Such a bursting of the incompatible pollen tube within the style has been reported by some authors in various genera such as Abutilon (Sears, 1937) and Coffea (Devreux, Vallaeys, Pochet & Gilles, 1959) and it is probable that the phenomenon
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is extremely widespread in self-incompatible species and results, as could be demonstrated in the present study, from a complete absence of the inner wall. It is interesting to note, in this connexion, that the swelling of the tube apex, reported by several authors to characterize the morphology of incompatible pollen tubes (Sears, 1937; Devreux et al. 1959; Kho & Baer, 1971) is not strictly related to the bursting process and is only due to considerable expansion of the outer wall which thickens to the extent of spreading against the neighbouring cells of the conducting tissue. The phenomena may not, however, be entirely unconnected because a slackening of growth due to abnormal wall metabolism at the tip of the tube could lead to both the expansion of the outer wall and the bursting of the tube. In spite of its fibrillar structure, the outer wall does not appear to contribute to the solidity of the tube and does not prevent its bursting.

These facts being established, important questions still remain to be answered which may be summarized in the following manner:

is the destruction of the pollen tube the cause or the consequence of growth-inhibition?

are the particles observed at the tip of the incompatible tube originating from the dismantling of the inner wall or do they result from the mobilization in the cytoplasm of material which is no longer allowed to participate in the biogenesis of the wall?

what is the relationship between the incompatibility reaction and the degradation process taking place in the incompatible tubes?

Whereas it is a difficult task to provide unequivocal answers to each of these 3 questions, sufficient elements appear to be available for initiating a rational discussion of the problem.

Function of the lytic phenomenon: since the compatible pollen tubes which have ceased to grow obviously open to release the spermat nuclei and to discharge the spherical particles at the level of the synergid, it would seem logical to consider that these events are a consequence of growth interruption. Should such be the case one could then suggest that the formation of particles in the incompatible pollen tube and its subsequent bursting are also a consequence of growth inhibition. Several reasons appear to militate, however, against such a conclusion. The first is that it is well established, according to Jensen & Fisher (1968) and to Vazart (1971), that the opening of the compatible pollen tube is induced by its passage through the degenerated synergid and not by a mere cessation of growth. A second reason is provided by the fact that there does not seem to be, for the incompatible pollen tube, a structural barrier, similar to the one met by the compatible pollen tube in the embryo sac, which could prevent its growth through the style. A third argument against the hypothesis that the destruction of incompatible pollen tubes arises as a consequence of growth inhibition is that it is difficult to visualize how this inhibition could lead to the thickening of the outer wall, the disappearance of the inner wall and the accumulation of particles in the tube cytoplasm. It therefore appears that the elimination of the inner wall and the bursting of the incompatible pollen tube are the causes and not the consequences of growth inhibition. This conclusion answers the question formu-
lated by Lewis (1965) and shows that self-incompatibility is not a passive process resulting from an absence of growth stimulation but an active phenomenon which leads to the destruction of the pollen tube.

Origin of the particles: it is possible, as stated earlier, that the elimination of the inner wall of the incompatible pollen tube and the subsequent bursting of the tube are due to the failure of the cytoplasm to contribute the material necessary for the biogenesis of the wall. On this assumption the numerous particles accumulated in the cytoplasm would represent building precursors of the inner wall which have lost their capacity to be integrated in the wall. The hypothesis is, however, somewhat unlikely because it could be observed, in a few instances, that the particles were included in the inner wall or, in some cases, present in the cytoplasm but still attached to the inner wall by a loose connexion which could hardly have been established if the particles had not originated from the tube wall. It is therefore probably more logical to consider that the disappearance of the inner wall results from its dismantling and from its breaking down into numerous particles which are released in the tube cytoplasm. As only the shell and not the core of the particles appears to be similar to the inner wall of the tube it is, however, probable that their formation involves both a basic constituent of the inner wall and some kind of synthetic activity.

Nature of the degradation process: as the processes involved in the degradation of incompatible pollen tubes appear similar to the ones operating in the compatible tubes which enter the degenerated synergid, it seems that the self-incompatibility reaction can be equated to an anticipation of the phenomenon scheduled to take place at the time the pollen tube has reached the ovule. Under such a hypothesis the S-complex would have to be considered as the inducer, in the style, of a reaction which normally occurs, after compatible mating, at the level of the micropylar region.

Whatever the correctness of this conclusion may be, the finding that the inner wall disappears at the apex of incompatible tubes certainly supports the current hypothesis (Mäkinen & Lewis, 1962; Linskens, 1965) that the tube wall is the site of action of the incompatibility protein. In all probability the identical S-alleles in pollen and style produce identical S-proteins which polymerize in the tube wall and combine with some constituents of the inner wall to produce the numerous particles accumulated in the tube cytoplasm. The nature of these particles is still unknown but some of their characteristics (size, shape, negative reaction to Thiéry's test, apparent ability to lyse the host cell) suggest that the incompatibility reaction presents some analogies with the processes leading to the lysis of virus-infected cells. Another observation indicating a possible resemblance between the 2 phenomena stems from the finding by Linskens (see Discussion in Lewis, 1965) that an RNA which is unique to the incompatibly pollinated styles is synthesized after pollination.

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REFERENCES


D. de Nettancourt and others


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Fig. 1. Cross-section of an unpollinated style of *Lycopersicum peruvianum*. The cells of the conducting tissue are completely separated from one another. \( \times 4480 \).
Fig. 2. Cross-section of the conducting tissue in the basal portion of a style pollinated with compatible pollen 24 h before fixation. The apical region of the pollen tube displays a circular cross-section and the tube wall is clearly bipartite. × 16 200.

Fig. 3. Cross-section of the conducting tissue in the upper portion of a style pollinated with compatible pollen 24 h before fixation. The cross-sections of the pollen tubes are flattened and generally present numerous infoldings; the tube wall is bipartite. × 15 000.

Fig. 4. Longitudinal section of the conducting tissue in the basal portion of a style pollinated with compatible pollen 24 h before fixation. The pollen tube wall is bipartite and numerous inclusions of different sizes and shapes are visible in the tube cytoplasm. × 16 200.
Fig. 5. Cross-section in the micropylar region of an ovule fixed 72 h after pollination with compatible pollen. Numerous particles which have a granular structure and sometimes appear to be surrounded by a less-electron-dense outer shell are visible in the degenerated synergid. × 15600.

Fig. 6. Cross-section of the conducting tissue in the upper region of a style pollinated with incompatible pollen 24 h before fixation. The inner-wall (inw), in the apical region of the incompatible pollen tube is not yet broken down into small particles and is clearly distinct from the outer wall (outw). The endoplasmic reticulum (er) shows a concentric parallel configuration. × 25800.
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Fig. 7. Cross-section of the conducting tissue in the upper region of a style pollinated with incompatible pollen 24 h before fixation. In the apical region of the pollen tube, the inner wall has disappeared and the tube cytoplasm is rich in isolated or aggregated particles with a granular core and a less-electron-dense outer shell. Double profiles (arrows) are frequently found along the edges of the particles. × 59,400.

Fig. 8. Thiery's test on incompatible pollen tubes. The outer wall (ow) of the tube and the particles only show a weakly positive reaction. The double membranes (arrows) lining some of the particles have reacted strongly. × 22,800.
Fig. 9. Cross-section of the conducting tissue in the upper region of a style pollinated with incompatible pollen 24 h before fixation. An incompatible pollen tube has burst open. The inner wall is absent and a great mass of particles are released in the intercellular space of the stylar tissue. ow, outer wall.  x 7840.

Fig. 10. Thiéry's test on the particles which have been released by an incompatible tube in the intercellular space of the conducting tissue. The particles are only slightly marked. Heavily marked starch (arrows) can be observed, for comparison purposes, in chloroplast of a stylar cell (sc). Note the lack of reaction of the inner wall (in) in the 2 portions of pollen tube.  x 22800.
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