

Ultrastructure of fertilization in *Plumbago zeylanica*

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Abstract

The synergidless female gametophyte of *Plumbago zeylanica* receives the pollen tube through specialized cell wall ingrowths at the base of the egg; tube growth continues between egg and central cells. Pollen tube discharge occurs between egg and central cell and results in release of two male gametes, vegetative nucleus, and some pollen cytoplasm. Except for the location of gamete discharge, details of transmission and fusion of gametic nuclei appear to conform to reports of these processes in taxa possessing conventional embryo sacs.

INTRODUCTION

The importance of synergids in receiving the pollen tube and physiologically mediating the transmission of sperm nuclei has been established by numerous electron microscopic studies (Jensen, 1973; Mogensén, 1978). To date, these studies have not provided any exceptions to the emerging concept that functional pollen tubes enter the embryo sac through one synergid and within that cell, discharge two sperm and a limited amount of pollen cytoplasm. Morphological changes within a single synergid appear to increase the likelihood that it will be penetrated by a pollen tube and may be required for passage of sperm nuclei into egg and central cells (Mogensén, 1978). It is therefore important to study the path of the pollen tube, its site of discharge, and the transmission of sperm nuclei in female gametophytes which lack synergids.

MATERIALS AND METHODS

Flowers of *Plumbago zeylanica* L. were artificially pollinated, collected 8 to 10 h later, and prepared according to standard electron

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Embryo sac structure and course of the pollen tube in *Plumbago zeylanica*
 Note: Figs 1, 2, 4, 5, 8-13 are oriented with the chalazal end of the embryo sac toward the top of the page. Figs 1-3, 6, 8-10 — transmission electron microscopy. Figs 4, 5 — phase-contrast microscopy. Figs 7, 11-13 — Nomarski differential interference contrast microscopy.

Fig. 1. The egg (below) and central cell of an unfertilized embryo sac. (Scale bar = 5 μ m)

Fig. 2. Lateral cell, embryo sac wall, and adjacent nucellus soon after fertilization

Fig. 3. Pollen tube (PT) within thick-walled transmitting tissue of lower style. (Scale bar = 5 μ m)

Fig. 4. Pollen tube approaching inner integument (located above pollen tube). Vegetative nucleus (arrowhead) and two sperm cells (arrows) are visible near pollen tube apex. (Scale bar = 5 μ m)

Fig. 5. Pollen tube (arrow) appressed against nucellus (Nc) after penetrating the micropyle (immediately below nucellus). (Scale bar = 10 μ m)

Fig. 6. Cross section through base of zygote with a flattened pollen tube in filiform apparatus shortly after fertilization. (Scale bar = 5 μ m)

Fig. 7. Cross section of upper egg wall soon after pollen tube discharge. Arrows point to cytoplasm of an unfused sperm cell between egg (above) and central cell (below). (Scale bar = 5 μ m)

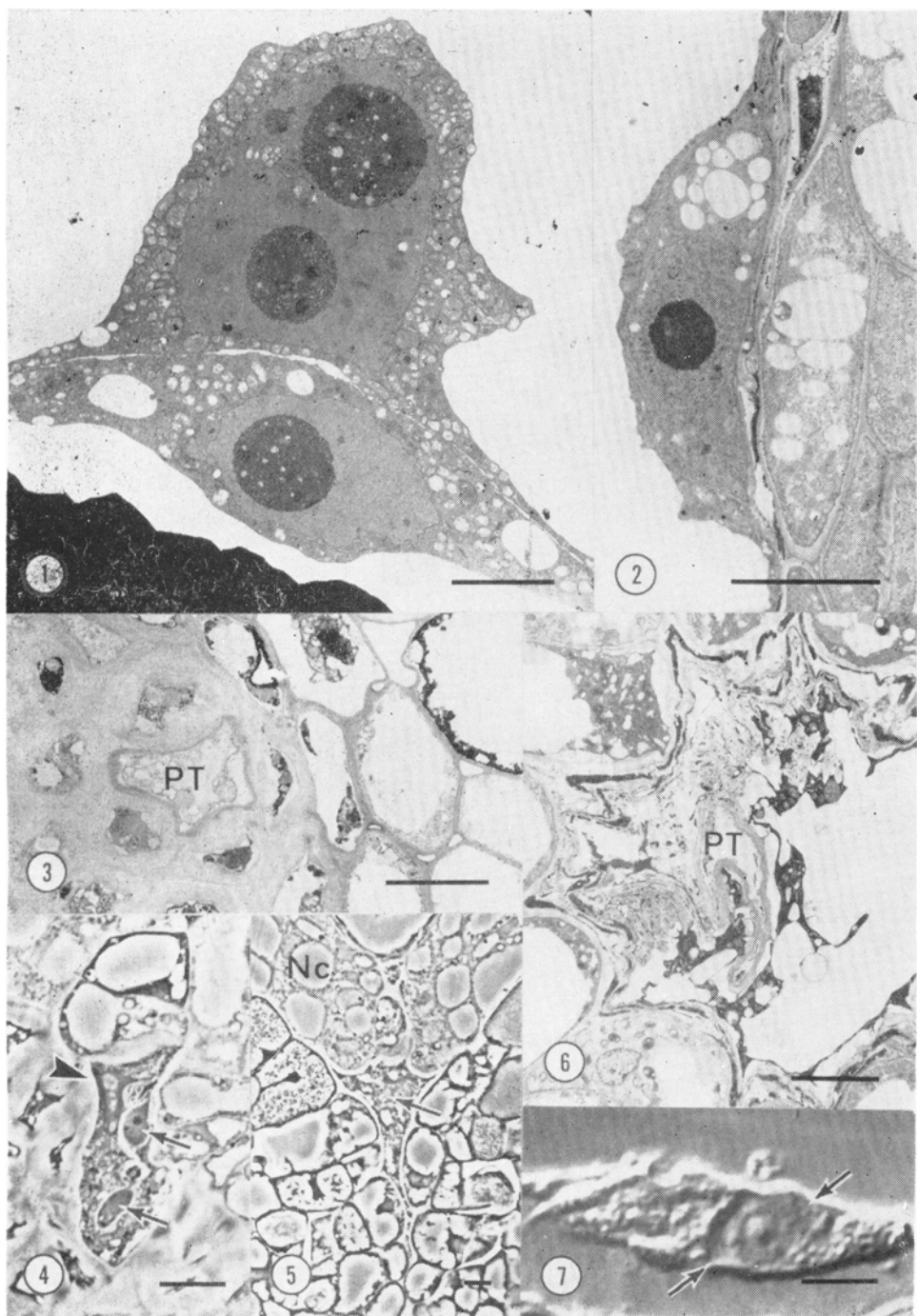
microscopic methods (Cass, Karas, 1974). Thin sections were stained with uranyl acetate and lead citrate (Figs. 1-3, 6, 10), or thiocarbohydrazide-silver proteinate reaction (Thiéry, 1967) with prior periodic acid oxidation (Fig. 8) or without (Fig. 9). Thick sections were observed prior to staining (Figs. 4, 5, 7) or after brief staining in 1% aniline blue black in 30% acetic acid (Figs. 11-13).

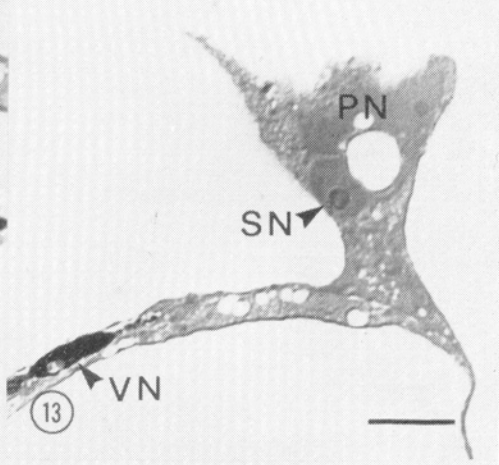
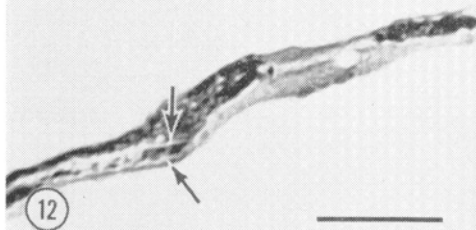
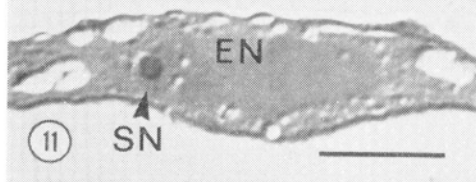
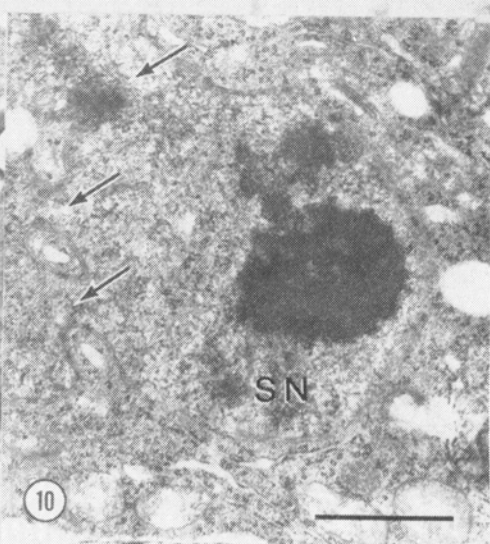
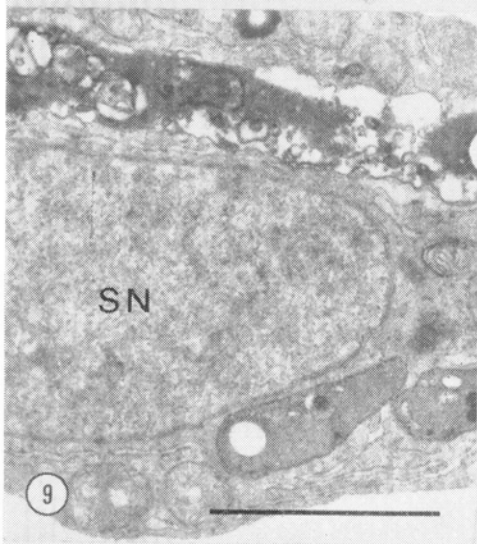
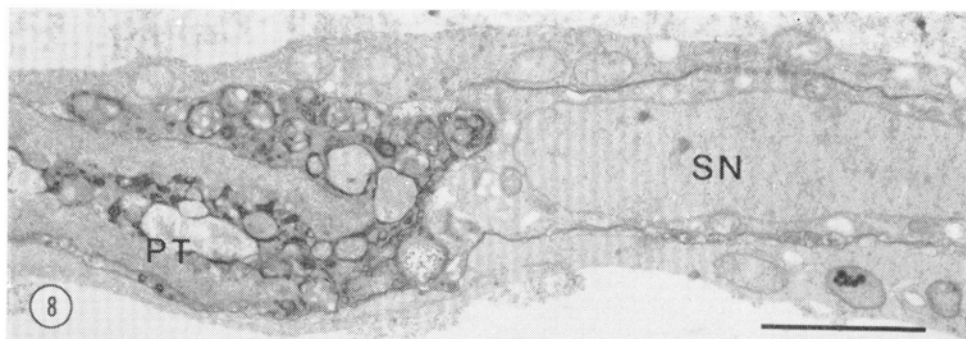
OBSERVATIONS

The unfertilized megagametophyte of *P. zeylanica* consists of five cells: the egg, central cell, and three accessory cells (two lateral cells and one chalazal cell). The mature egg is a highly polarized cell occupied by a prominent central vacuole. The nucleus, numerous mitochondria, and a majority of the plastids are located at the chalazal end (Fig. 1), while the micropylar end of the egg possesses cell wall ingrowths (Cass, Karas, 1974). The central cell nucleus, produced by the fusion of polar nuclei, is situated opposite the egg nucleus (Fig. 1). Its cytoplasm is largely perinuclear and is linked by narrow strands to a thin layer of cytoplasm (Fig. 2) bordering the central cell. Accessory cells are attached to the periphery of the embryo sac (Fig. 2). Although these cells have no obvious function, they may persist during embryogenesis without morphological alteration.

Following pollination and initial penetration of the pollen tube into the stigma, tube growth occurs exclusively in thick cell walls of the closed stylar transmitting tissue (Fig. 3). In the pollen tube, the vege-

PLATE I





Embryo sac of *Plumbago zeylanica* near double fertilization

Note as in Plate I.

Fig. 8. Sperm nucleus (SN) within central cell soon after nuclear transmission. Pollen tube (PT) aperture, shown between the egg and central cell plasma membranes, indicates location of pollen tube discharge. (Scale bar = 2 μ m)

Fig. 9. Sperm nucleus (SN) within egg of the embryo sac as shown in Fig. 8. (Scale bar = 2 μ m)

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Fig. 10. Sperm nucleus (SN) forming nuclear bridges with the egg nucleus (arrows) during fertilization. (A serial section is shown in Fig. 11). (Scale bar = 2 μ m)

Figs 11-13. Serial thick sections showing the salient features of double fertilization in a single embryo sac. (Scale bar = 10 μ m)

Figs 11-13. Serial thick sections showing the salient features of double fertilization.

Fig. 12. Pollen tube aperture (arrows) is contrasted against the densely stained discharged pollen cytoplasm located between incipient zygote (below) and endosperm (above)

Fig. 13. Sperm nucleus (SN) fusing with central cell nucleus (PN). The vegetative nucleus (VN) is visible between zygote and endosperm

tative nucleus and two sperm cells approach the growing tip as the tube nears the ovule (Fig. 4). Pollen tubes enter the ovule at the micropyle and displace several nucellar cells before entering the embryo sac (Fig. 5). Pollen tubes penetrate the female gametophyte through a filiform apparatus at the base of the egg (Fig. 6) and grow for another 80 μ m between egg and central cell. Pollen tube discharge occurs through a terminal aperture, resulting in release of male gametes, vegetative nucleus, and a limited amount of pollen cytoplasm (Figs. 8, 12, 13). Discharged male gametes remain cellular during their brief presence between egg and central cells (Fig. 7), and ultrastructurally, their cytoplasmic organelles appear viable.

Male gametes are tightly appressed to both egg and central cells (Fig. 7); however, only one sperm nucleus is transmitted into the egg (Fig. 9), and one sperm nucleus into the central cell (Fig. 8). Electron microscopic observation of these cells soon after nuclear transmission strongly suggests that sperm cytoplasmic organelles are also transmitted during the process of gamete fusion (Russell, 1980). Unfused sperm nuclei are briefly present within cells of the female gametophyte and migrate up to 30 μ m to align with their counterpart nuclei. Nuclear fusion occurs by the formation of nuclear bridges between male and female nuclei (Figs. 10, 11, 13) and appears to be completed before division of the zygote (Russell, Cass, unpublished).

DISCUSSION

Conventional embryo sac function appears to require the involvement of synergids to mediate transmission of sperm nuclei into the egg and central cells (Jensen, 1973). Since this concept has figured prominently in recent literature, the absence of synergids in four genera of the *Plumbagineae* (Cass, 1972) prompts the question of whether double fertilization, in the conventional sense, can occur in synergidless angiosperms. In the only previous observation of fertilization in the *Plumbagineae*, Dahlgren (1916) identified and illustrated sperm nuclei in both egg and central cells of *Plumbagella micrantha*, but apparently did not observe nuclear fusion in the central cell. Dahlgren (1916, p. 61) concluded: "So genannte Doppelbefruchtung findet wahrscheinlich statt". Observations of sperm nuclei transmitted into egg and central cells of *Plumbago zeylanica*, and nuclei subsequently fused to constitute the zygote and primary endosperm nucleus, indicate that double fertilization in *Plumbago* is essentially similar to that reported in other angiosperm taxa.

We believe that the embryo sac of *P. zeylanica* exhibits a highly specialized and reduced egg apparatus consisting of a single modified egg cell. When compared to typical egg apparatuses (Jensen, 1973), the ultrastructural organization of the egg of *P. zeylanica* resembles that of a synergid (Cass, Karas, 1974). The presence of a filiform apparatus, the ultrastructural appearance of a physiologically active cell, and the extreme micropylar position of the egg are all bases for comparing the egg of *Plumbago* with a synergid (Cass, 1972; Cass, Karas, 1974). Recent discoveries that the pollen tube enters the embryo sac through a filiform apparatus at the base of the egg and that the egg provides a specific location for pollen tube discharge and gamete fusion reinforce these similarities. Major differences between this egg and a synergid include: 1) the egg of *Plumbago* does not directly receive the pollen tube; 2) there is no visible alteration of egg cytoplasm required for pollen tube growth or discharge, or for passage of sperm nuclei; 3) the egg functions as a gamete and gives rise to a normal embryo.

The important functions of an egg apparatus, namely receiving the pollen tube and serving as depositional site for two sperm cells, are relegated to an intercellular location in *P. zeylanica*. The absence of recognizable degenerative changes in the egg of *P. zeylanica* contrasts with the system reported in the majority of conventional embryo sacs where synergid degeneration precedes, accompanies, or follows pollen tube penetration into the embryo sac. The precise role, if any, of synergid degeneration in conventional embryo sacs is unknown, but suggestions about release of chemotropic substances to attract the pollen

tube (Pluijm, 1964) and the ability to modify sperm cells (Mogensen, 1978) have been made. It is possible that pollen tube chemotropism and sperm cell modification occur during the fertilization process in *P. zeylanica*, but the probable sites involved in mediating these events must await further investigation.

Acknowledgments

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