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GAMMA IRRADIATION OF PRUNUS AVIUM L. FLOWER BUDS: EFFECTS ON STYLAR DEVELOPMENT – AN ULTRASTRUCTURAL STUDY*

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SUMMARY

An attempt has been made to analyse the effects of acute gamma irradiation on stylar development in *Prunus avium*. The treatment was applied to meiotic buds. The observations with electron microscopy were made on styles collected from the flowers one day after anthesis. The data showed that in the irradiated plants, some cells of the stigma were partially degenerated. The transmitting tissue in the style contained not only the cells with normal cytoplasm, but also the cells having cytoplasm at different stages of degeneration. Empty zones, similar to vacuoles, are present in the intercellular stylar substance, probably due to poor secretion activity of the cells. Further, in the irradiated plants the cells of the tramsmitting tissue contained exclusively free ribosomes and there was considerable decrease in the activity of RER and Golgi bodies indicating a decreased synthetic cellular activity of the transmitting tissue. The possible implications of the radiation effects on the stylar transmitting tissue in relation to the incompatibility mechanism are discussed in the light of the results obtained in the present study.

1. INTRODUCTION

In an attempt to obtain self-compatibility mutations in the prominent Italian sweet cherry cultivars, and particularly in the desirable morphological mutants ("spur mutants") maintained at the Nuclear Centre of Casaccia near Rome, investigations are carried out on the effects of gamma rays on flower buds.

In *Prunus avium* L. (sweet cherry) the self-incompatibility mechanism is based on a homomorphic gametophytic system controlled by multiple alleles at one locus and causes the inhibition of pollen tubes half-way down the style (CRANE & LAWRENCE 1931; CRANE & BROWN 1937; ROY 1939; see also LEWIS 1941). In the past various physical and chemical methods have been tried suc-

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cessfully to induce temporary or permanent self-compatibility in many different plant species (for a review see DE NETTANCOURT 1969, 1972, 1977) and the treatment with ionizing radiations has often been demonstrated as an efficient technique for inducing self-compatibility mutations in higher plants (LEWIS 1951, 1954, 1960; PANDEY 1956, 1965, 1969, 1970; BREWBAKER & NATARAJAN 1960; VAN GESTEL & DE NETTANCOURT 1974, 1975; DE NETTANCOURT & ECOCHARD 1968). Also in *Prunus avium* LEWIS (1949) and LEWIS & CROWE (1953, 1954) have reported the induction of self-compatibility mutations after X-ray-irradiation of pollen mother cells.

In our study cherry plants belonging to the cultivar "Mora di Cazzano" have been treated with gamma irradiation. The choice of the dose was that previously used by LEWIS (1949). Data on the overcoming of the incompatibility reaction shall be reported at a later date; in this first article the results are presented of a comprehensive morphological investigation on treated styles.

2. MATERIAL AND METHODS

The plants of *Prunus avium* L. cultivar "Mora di Cazzano" grown in large pots under isolation at Casaccia Nuclear Centre near Rome, were used for irradiation treatments with gamma rays when the pollen mother cells were undergoing meiosis. It is known from the experiments by LEWIS (1949) in *P. avium* L. and in *Oenothera organensis* that self-compatibility mutations can not express themselves in the pollen and can not be detected in the treated generation, if irradiation is applied after meiosis.

Gamma irradiation was carried out with a 67000 Ci ^{60}Co source at the Casaccia Nuclear Centre. The choice of the dose, 800 rad was based on its effectiveness in inducing compatibility mutations in *P. avium* (see LEWIS 1949). The dose rate given was 450 R per minute. The temperature during irradiation was 22° ± 1 °C.

2.2. Electron microscopy

The styles of *P. avium* collected from the flower one day after anthesis, were transversely cut into three segments in order to have a more uniform fixation. The samples were fixed in 3% glutataldehyde in 0.066 M cacodylate buffer, pH 7.2 for 2 h at room temperature. They were rinsed in the same buffer and post-fixed in buffered 1% OsO_4 for 1 h.

Dehydration was carried out in ethanol with a last passage in propylene oxide, and the material was embedded in an Epon-Araldite mixture. Sections were cut with a LKB ultrotome III using a glass knife, post-stained with lead citrate and uranyl acetate and examined with a JEOL JEM 100 B at 60 kV.

3. RESULTS

3.1. Non-irradiated plants: ultrastructural features of the stylar transmitting tissue

In cross sections the mature style of *P. avium* appears round with a central transmitting tissue and with several peripheral layers of vacuolized cells. The cells of the transmitting tissue are also round (*fig. 1*); they are partially rich in amyloplasts, containing 2 to 7 large starch grains; the round nucleus shows only one big nucleolus. The dictyosomes are particularly abundant and produce many vesicles; the endoplasmic reticulum (ER) is of the rough type and its long profiles are located near the plasma membrane and the nuclear envelope. Rough endoplasmic reticulum (RER) enlargements are present and dictyosome vesicles can be frequently observed in proximity or in contact with the plasma membrane (*figs.* 2–3). Most of the ribosomes are gathered in polysomes, often located near the plasma membrane (*fig. 3*). The cytoplasm contains also mitochondria, small vesicles and lipid bodies. The cell walls do not show plasmodesmata; the cells appear in fact isolated and immersed in the strongly electron-dense, fairly uniform, intercellular substance through which the pollen tubes grow towards the ovary. In longitudinal sections the cells of the transmitting tissue have the usual appearance already observed in other solid styles (Cresti et al. 1976); they are elongated and rectangular, with nuclei also elongated and many plasmodesmata in the transverse walls.

In the mature style the cells of the transmitting tissue continue to secrete the intercellular substance, which at first forms small remaining attached to the cell wall (*fig. 4*). At this stage the intercellular substance is compact and electrondense.

It is noted that also the stigmatic cells continue to produce stigmatic exudate.

3.2. Irradiated plants: ultrastructural features of the stylar transmitting tissue

The style of the irradiated plants has externally the same characteristics as that of the non-irradiated plants.

The transmitting tissue shows two different types of cells: 1) cells with apparently normal cytoplasm, but with apparently weakly active organelles, 2) cells with cytoplasm at different stages of degeneration (*fig. 5*).

The cellular layers surrounding the transmitting tissue do not show any relevant modification in comparison with the control styles.

Type 1 cells as seen in cross-sections are round and contain amyloplasts with 1 to 4 starch grains, few RER profiles, weakly active dictyosomes, mainly free ribosomes, mitochondria and large vacuoles. In the nucleus the chromatin is weakly electron-dense and only one small nucleolus is present; the cell walls do not contain plasmodesmata.

The cellular degeneration process seems to start when stacked profiles of membranes appear (*fig. 6*) in the wall, outside the plasmalemma, which loses its connection with the cell wall (*fig. 7*). In this way cytoplasm contraction

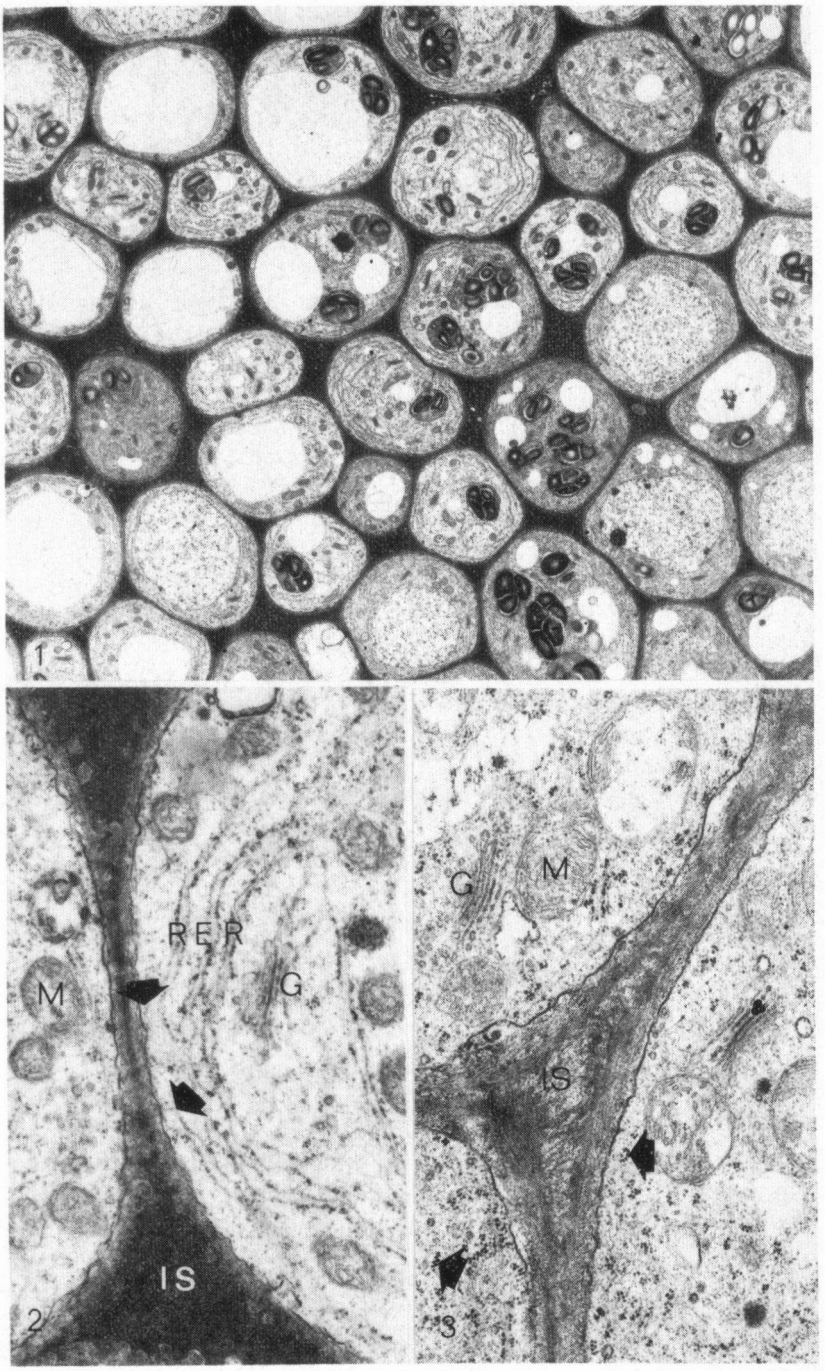

Fig. 1. General aspect of the stylar transmitting tissue of the non-irradiated plants. × 3100.
Fig. 2. Non-irradiated plants. Rough Endoplasmic Reticulum (RER) enlargements near the plasma membrane (arrows). G : Golgi body; M : Mitochondria; IS : Intercellular Substance. × 18000.
Fig. 3. Golgi body (G), polyribosomes (arrows) in proximity of the plasma membrane in non-irradiated plants. × 20000.

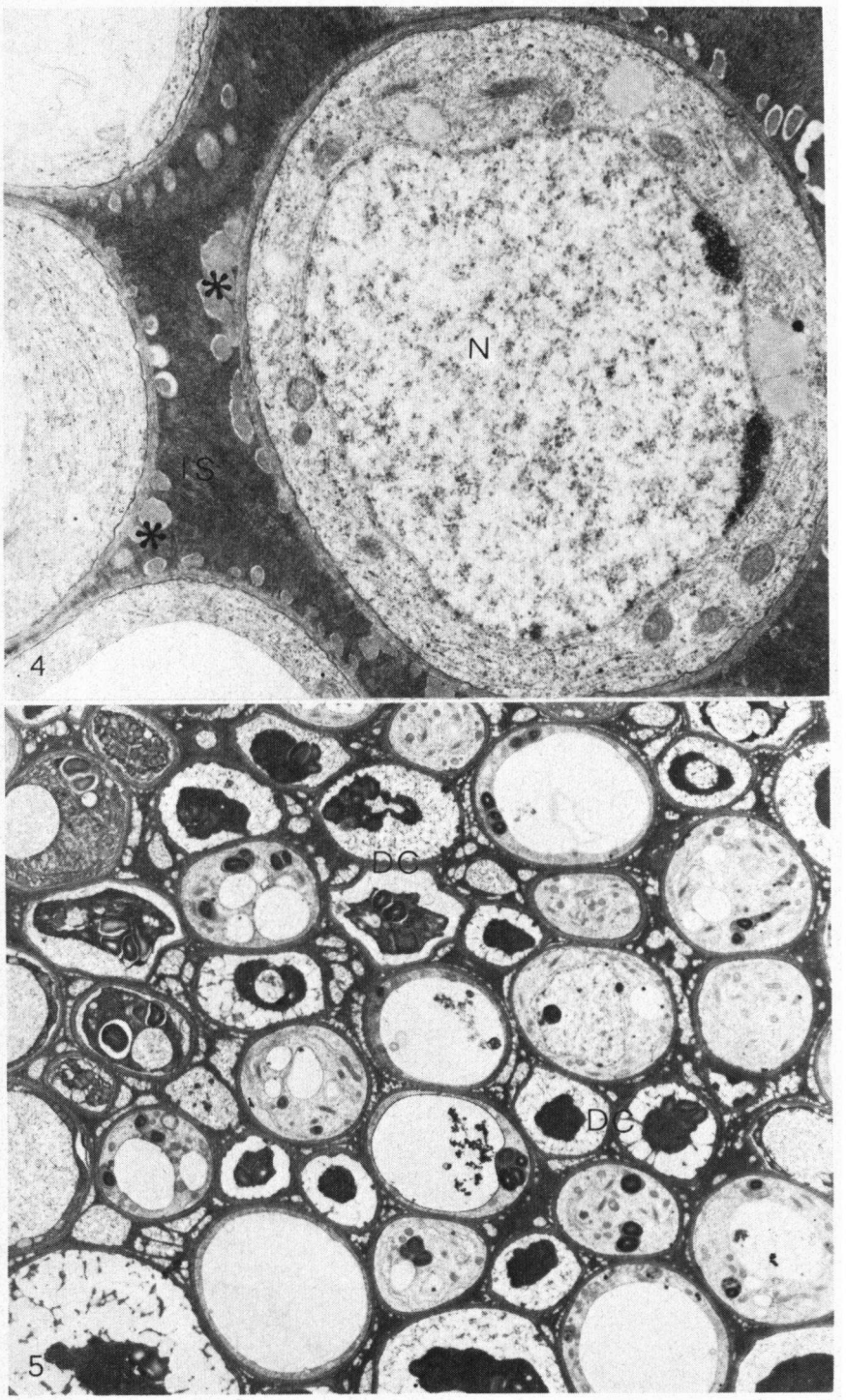

Fig. 4. Non-irradiated plants. Small masses of intercellular substance are attached to the external cell wall (asterisks). N : Nucleus. × 11400.

Fig. 5. Irradiated plants. General aspect of the stylar transmitting tissue. Many empty areas are present in the intercellular substance. DC: Degenerated Cell. × 3200.

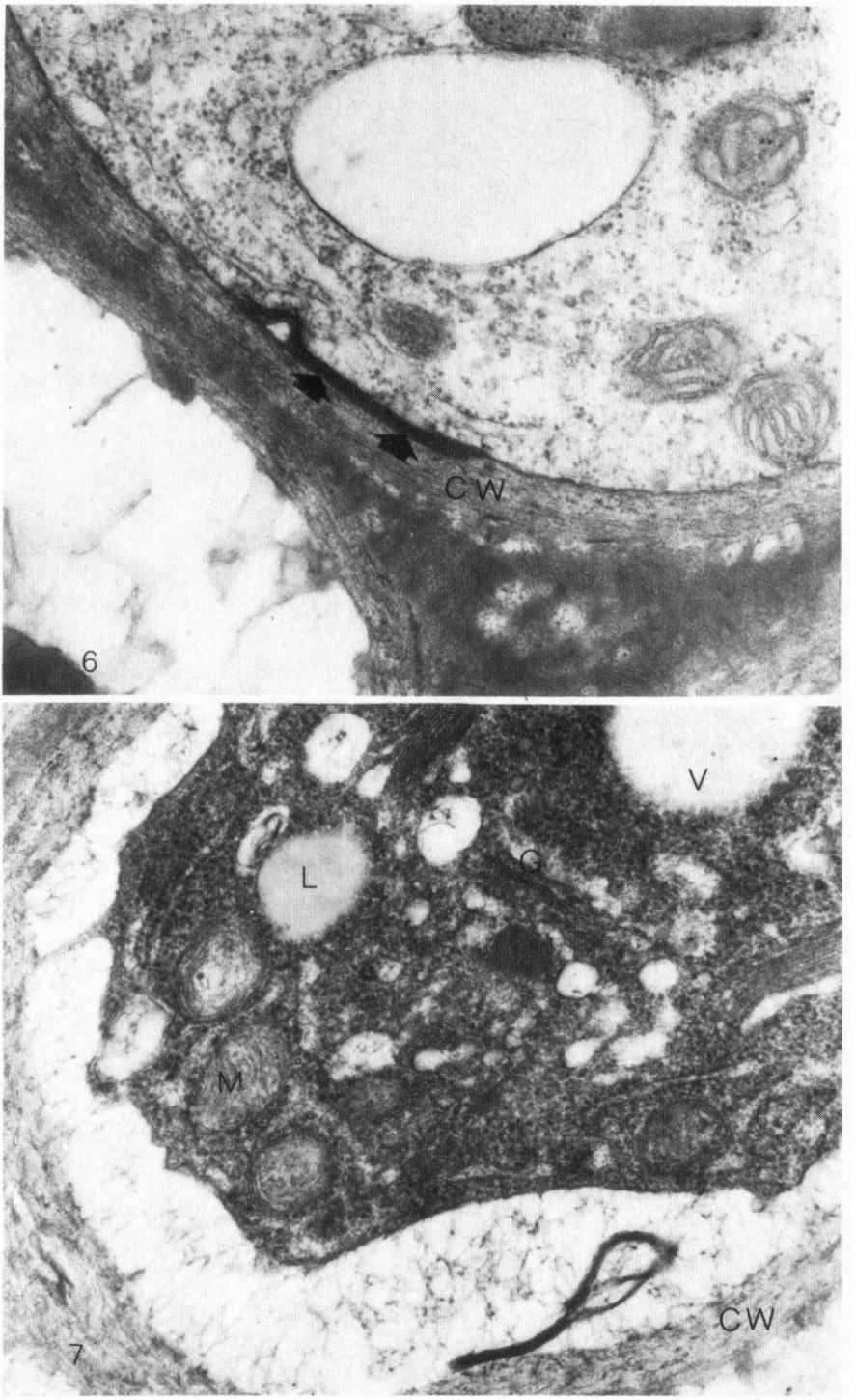

Fig. 6. Beginning of the cellular degeneration process of the stylar transmitting tissue. Stacked profiles of membrane appear below the plasma membrane (arrows). CW : Cell Wall. × 36000.

Fig. 7. Disconnection of the plasma membrane from the cell wall: all cellular organelles are still evident in the cytoplasm. L : Lipids; V : Vacuoles. × 36000.

occurs as if a plasmolysis process took place (*fig. 7*). At this degeneration stage all the cellular organelles are still evident in the cytoplasm. Fibrillar material is present in the region between cytoplasm and cell wall. The cytoplasm quickly degenerates forming a black central mass where only starch grains are visible (*fig. 8*). The final stage of this process occurs when the black mass is considerably decreased and the region between the cytoplasm and the cell wall is filled with weakly electron-dense material (*fig. 9*).

The intercellular substance surrounding both normal and degenerated cells shows some fairly homogeneous electron-dense zones together with scattered empty areas, similar to vacuoles (*figs.* 5–8).

Neither normal cells nor cells in different degeneration stages secrete any intercellular substance.

In the stigmatic tissue, the cellular degeneration process shows the same features as described for the transmitting tissue. However, the degenerating cells are more abundant than those of the transmitting tissue, and the intercellular spaces, which should be filled with stigmatic exudate, show completely empty wide zones (*fig. 10*).

4. DISCUSSION

Gamma rays treatments of *P. avium* L. buds during meiosis cause damage both to the cells and to the intercellular substance of the stylar transmitting tissue. The irradiated cells, in particular, have a reduced number of starch grains; their ribosomes are almost exclusively free and the RER and Golgi bodies show a considerably reduced activity. This could indicate a decreased synthetic activity of the cells, particularly as far as proteins and polysaccharides are concerned, leading to a complete cessation of the secretion of intercellular substance.

LINSKENS et al. (1960) demonstrated that acute irradiation of *Petunia* styles, immediately before selfing, could attenuate the capacity of the style to reject incompatible pollen. HOPPER & PELOQUIN (1968) reported similar results for *Lilium longiflorum*. These authors underlined that the results obtained after style irradiation were very similar to those observed after heat treatment (BALI 1963; HECHT 1963; TOWNSEND 1965, 1966, 1968; HOPPER et al. 1967) and suggested enzyme inactivation as a possible cause of the reduced self-incompatibility; involvement of enzymes in the incompatibility reaction has been suggested by several authors (BREDEMEYER & BLAAS 1975; LINSKENS et al. 1969; SCHLÖSSER 1961). These enzymes, or more generally the proteinaceous fraction of the intercellular substance, probably playing a role in the incompatibility reaction (LINSKENS 1961, 1975), is formed, at least in *Lycopersicum peruvianum*, during the last stage of style development (CRESTI et al. 1976). In the irradiated *P. avium* styles the formation of the intercellular substance ceases just when it should accumulate the specific components responsible for the incompatibility reaction. To what extent this radiation damage of meiotic buds affects the self-incompatibility reaction is yet to be ascertained.

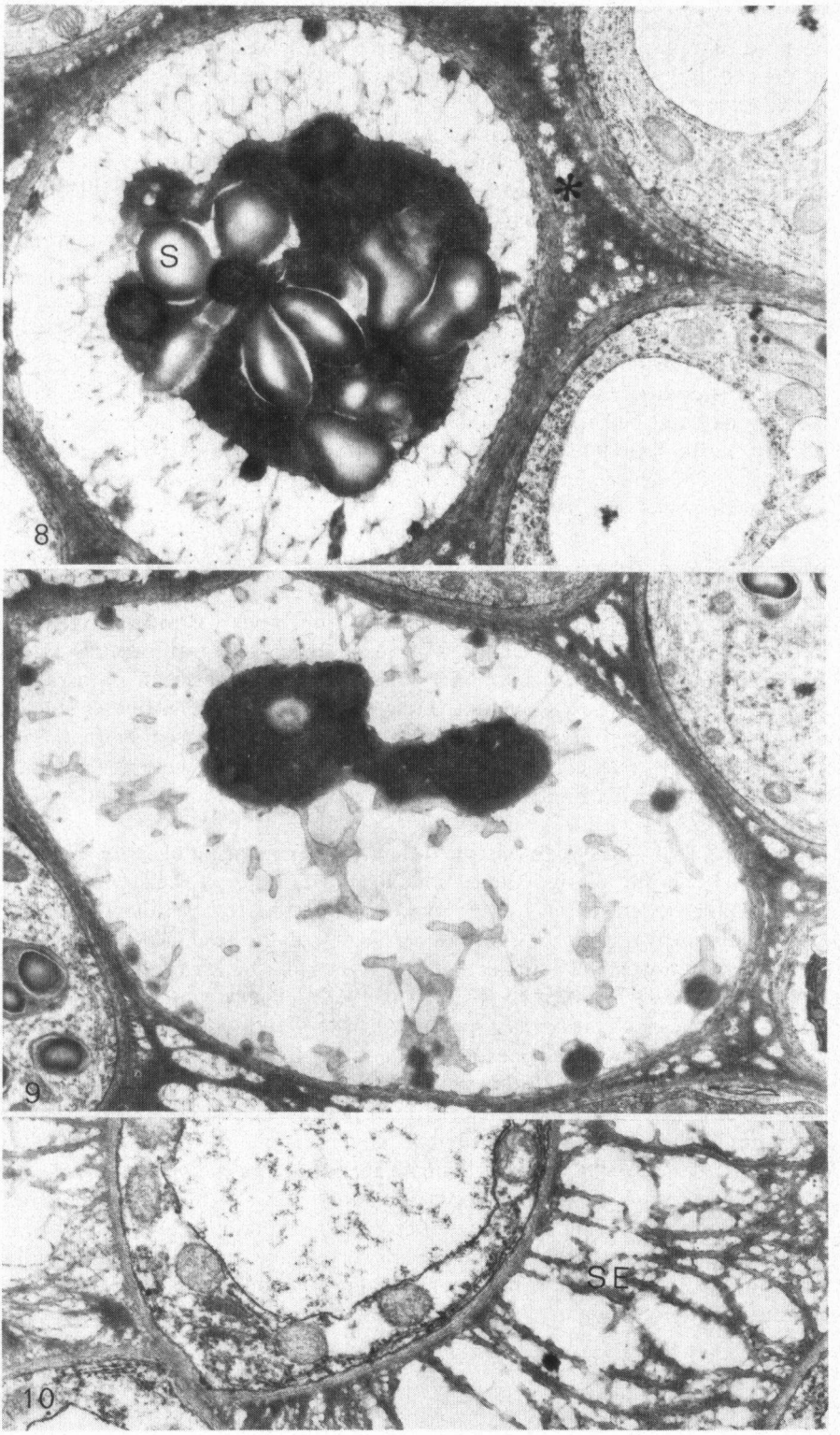
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Fig. 8. Degenerated cytoplasm of stylar transmitting tissue cells. Formation of a black central mass. Only starch grains (S) are visible. Many empty areas are present (asterisks) in the intercellular substance. × 12000.

Fig. 9. Final stage of degenerative process: PAS positive material occurs in the region between the degenerated cytoplasm and the cell wall. × 12000.

Fig. 10. Irradiated plants. Stigmatic tissue: the stigmatic exudate (SE) shows completely empty zones. × 18000.

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