EFFECTS OF GAMMA IRRADIATION ON ANTHER, MICROSPOROGENESIS AND MICROGAMETOGENESIS ON *SOLANUM NIGRUM* L. COMPLEX

P. S. CHANDEL

Department of Botany, J. N. Degree College, Lucknow, India

Abstract

Effects of varying acute doses of gamma rays (10, 20, 30, 40, 50, 60, and 70 k rads) on anther, microsporogenesis and microgametogenesis on diploid, t traploid and hexaploid *Solanum nigrum* L. complex have been studied.

Stamens are five in number. Anther is tetrasporangiate in control and most of the treatments, but anomalies have been noticed in number, shape and position of anthers at higher doses. In many cases of treatments, the anther lobes are seen undeveloped or poorly developed or completely sterile. Sometimes degenerated as well as fertile locules are present in the same anther. Anther wall development is similar to control though a few variations occurred in treatments. Abnormalities is seen in the tapetal cells in irradiation treatment. In some cases, the tapetal cells persisted for a longer time, while in a few cases they degenerated earlier. Highly enlarged and vacuolated tapetal cells are seen in a few anther lobes of tetraploids at higher doses. Cytokinesis is of the simultaneous type, but in several cases of treatments cytokinesis did not occur. Beside normal tetrads, formation of dyads, triads and polyads was observed in several treatments. In a few treatments compound pollen grains were formed. Pollen grains were generally tricolpate but pollen grains with one or four germ-pores were seen in various treatments in all the ploidy groups. Dehiscence of the anther takes place through the formation of resorption tissue. In several cases of treatments the resorption tissue was considerably reduced.

Introduction

Ionizing radiations have been increasingly used to study various biological aspects of plants such as, germination, gross morphology, genetics and mutation breeding but only a few workers have used ionizing radiations to investigate various aspects of embryology (Singh & Gunckel, 1965; Chauhan, 1968; Chopra & Singh, 1974). Since the information on the embryological aspects is missing in plants specially in complex group, the present investigation was undertaken to study the embryological changes specially on anther, microsporogenesis and microgametogenesis in response to various doses of gamma rays in *Solanum nigrum* L. complex.

Material and methods

Dry (moisture content 11%) and soaked (moisture content 100%) seeds of diploid, tetraploid and hexaploid *Solanum nigrum* were irradiated at Radiation Biology Laboratory of National Botanical Research Institute, Lucknow with the help of CO^{60} source emitting 1000 rads/75 seconds. The acute gamma-ray exposures used were 10, 20, 30, 40, 50, 60 and 70 k rads. 300 seeds were taken for each treatment. The irradiated seeds were sown in sterile petriplates, pots and beds. At flowering time different stages of flower buds were fixed in formalin-acetic alcohol and stored in 70 per cent ethanol. Material was dehydrated in tertiary butyl-alcohol series and embedded in paraffin wax in usual way. Serial microtome sections were cut at thickness ranging from 10 to 20 μm. Safranin-fast green combination was used for staining. Pollen sterility was tested by acetocarmine and iodine solution taking fresh flowers from the experimental plot.

Observations

Young anthers nearly oval in outline

in cross section (Text-fig. 1A), consist of densely cytoplasmic parenchymatous cells enclosed by a layer of epidermis. Soon, indication for four lobes becomes distinct (Text-fig. 1B) and at about this stage, a few cells in the hypodermal region become distinct in each lobe and function as the archesporial cells (Text-fig. 1C). The cells subsequently divide periclinally to form the cells of the primary parietal layer on outside and the cells of the primary sporogenous layer on the inner (Text-fig. 1D). Cells of the former undergo another periclinal division forming two cell-layers (Text-fig. 1E). The cells of the inner one differentiate as the tapetal layer, whereas those of outer layer undergo one more periclinal division forming two layers. The outer layer functions as the endothecium, whereas the inner forms the middle layer (Text-fig. 1F). The cells of the middle layer further divide periclinally resulting in two middle layers (Text-fig. 1H.) Sometimes, one more periclinal division leads to the formation of three middle layers (Text-fig. 1J). The anther wall thus consists of a layer each of epidermis, endothecium and tapetum and two or three middle layers.

The epidermis is persistent. The endothecium degenerates or collapses in most part of the mature anther, but its cells remain persistent in the apical region of the anther where they elongate radially and develop usual fibrous thickenings (Text-fig. 1Y).

Out of the two middle layers the cells of the inner one flatten and degenerate early (Text-fig. 1I, J) whereas those of outer middle layer usually persist longer (Text-fig. 1S).

Cells of the tapetum are uninucleate to begin with, but they soon become bi-, tri- or multinucleate by the time microspore tetrads are formed (Text-fig. 1S). The tapetal cells begin to degenerate by the time of microspores are formed. The tapetum is of the glandular type.

Variations in the number of stamens per flower and anomalies in the relative position of the anther lobes, their shape and number per anther have also been seen in irradiated material and the frequency of such anomalies increased with increasing doses though frequencies of such abnormal anthers is less in hexaploids as compared to diploids and tetraploids and the data are presented in Table 1.

Table 1—Anomalies in number of anther lobes

Treatment	No. of stamens examined	Diploid	Tetraploid	Hexaploid
Control	100	×	×	×
10 k rad	100	6	14	4
20 k rad	100	8	12	4
30 k rad	100	14	16	6
40 k rad	100	8	22	×
50 k rad	100	×	26	×

Details of variations observed in irradiated material are given below:

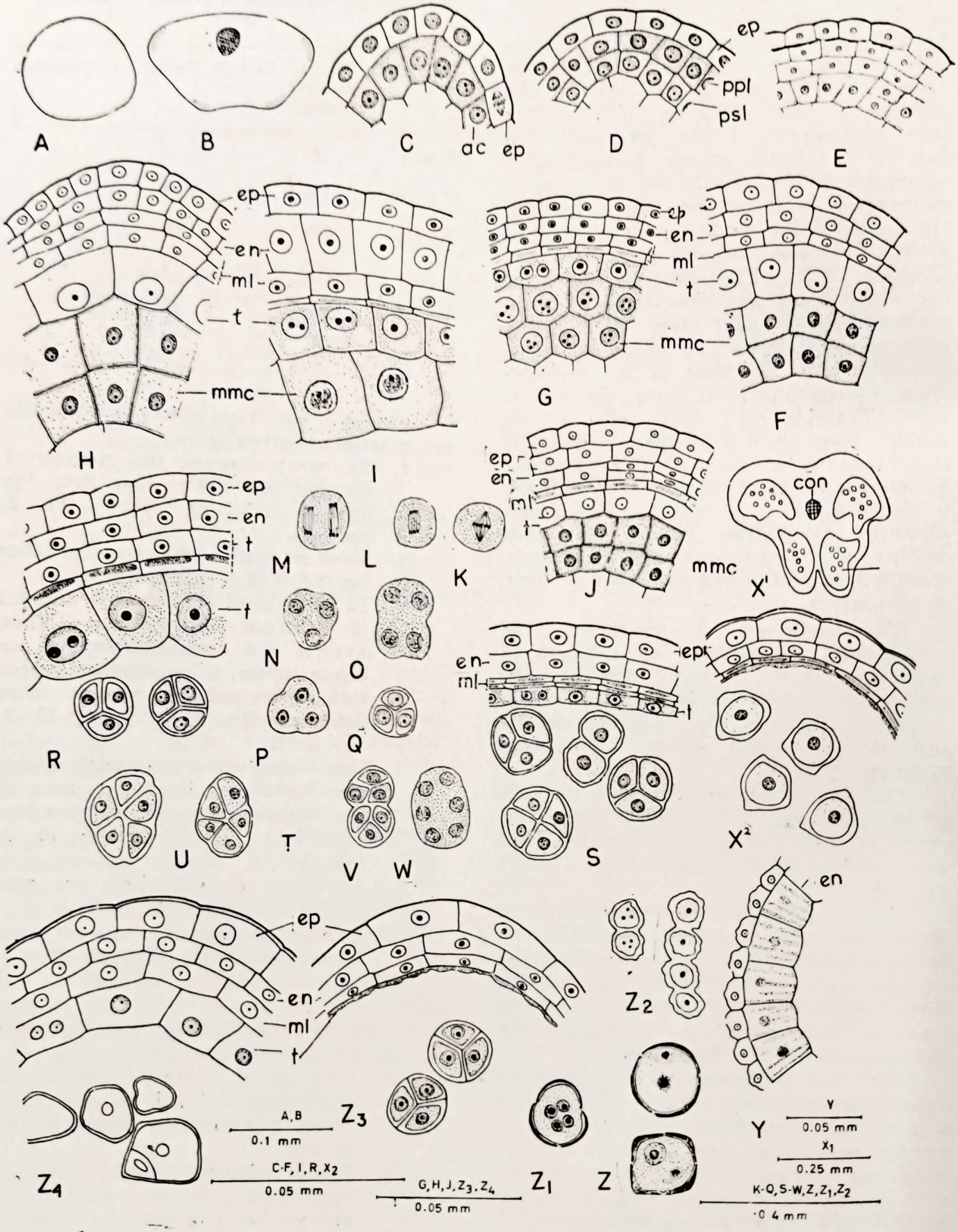
1. In many flowers, the number of stamens are more than five, the number reaching seven (Pl. 2, figs. 1-2).
2. Some stamens have five anther lobes instead of four (Pl. 2, figs. 4).
3. In many cases, anthers are unusual in structure and position. Some anthers are monosporangiate or bisporangiate, a few show only three anther lobes and some showed more than five lobes (Pl. 1, fig. 4; Pl. 2, figs. 2, 4).
4. Sometimes two or more anther lobes are fused and in a few cases all the anthers showed as syngenesious conditions (Pl. 1, fig. 3; Pl. 2, figs. 4).
5. In many cases anthers are seen arising from petals (Pl. 2, fig. 2)
6. In a few cases all the anthers have been found sterile (Pl. 2, fig. 3).

The general pattern of anther wall development in treatments is like that as described for the control, but following variations have been observed:

(a) Absence of fibrous thickening on the endothecial cells in the region where it is normally present in the control.

(b) Total collapse of middle layers, sometimes all the wall layers except the epidermis are seen to degenerate completely.

(c) Abnormalities in the tapetal cells at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X₁
0.25 mm
K-Q, S-W, Z, Z₁, Z₂
0.4 mm

Text-fig. 1

higher doses (30, 40, 50 k rad) are observed more in the diploids and tetraploids. In some cases, the tapetum degenerated at the microspore tetrad stage (Text-fig. 1Z_3), a stage where it is distinctly present in unirradiated material (Text-figs. 1R, S). In other cases this layer persisted even at the pollen grain stages (Pl. 1, figs. 1-2; Text-fig. Z_4). In other cases, a few anther lobes showed tapetum while in one or two lobes of the same anther it is degenerated (Pl. 1, figs. 2). The tapetal cells become highly vacuolated and sometimes enormously large.

Another interesting feature observed is the separation of tapetal cells from anther wall layers and their intermingling with the tetrads or microspores.

Microsporogenesis and microgametogenesis

The primary sporogenous cells which are arranged in a layer in the beginning undergo periclinal division to become two layered in all the three ploidy groups (Text-figs. 1E, H, J). These cells function as microspore mother cells and undergo meiosis resulting into four nuclei (Text-figs. 1K, L, M). Cytokinesis is of the simultaneous type and takes place by furrowing which grows centripetally resulting in the formation of tetrads of microspores which are arranged in tetrahedral, isobilateral or decussate manners (Text-figs. 1N, O, P, Q, R, S, T); the tetrahedral pattern being most common. Beside tetrads, formation of dyads, triads and polyads to a limited extent has also been observed in all the ploidy series (Text-figs. 1U, V, W, Z_2). Failure of cytokinesis has been seen at higher doses in all the ploidy groups (Text-fig. 1Z).

The microspores soon fall apart from the tetrad and develop into uninucleate pollen grains (Text-figs. 1 X_1, X_2) which are somewhat spherical in shape. During further development, a large vacuole is formed and the nucleus is pushed aside close to the wall. The nucleus now divides forming a small generative cell and a large vegetative cell (Text-fig. 1Z). Thus, at the time of dehiscence the pollen grains are two-celled. The pollen grains are spherical and smooth-walled. Sometimes they appear almost triangular or quadrangular in outline (Text-figs. 1Z). Pollen grains have usually three germpores.

General pattern of microsporogenesis and microgametogenesis in most of the treatments was similar to control.

Anther dehiscence

Dehiscence of the anther takes place by the formation of resorption tissue which

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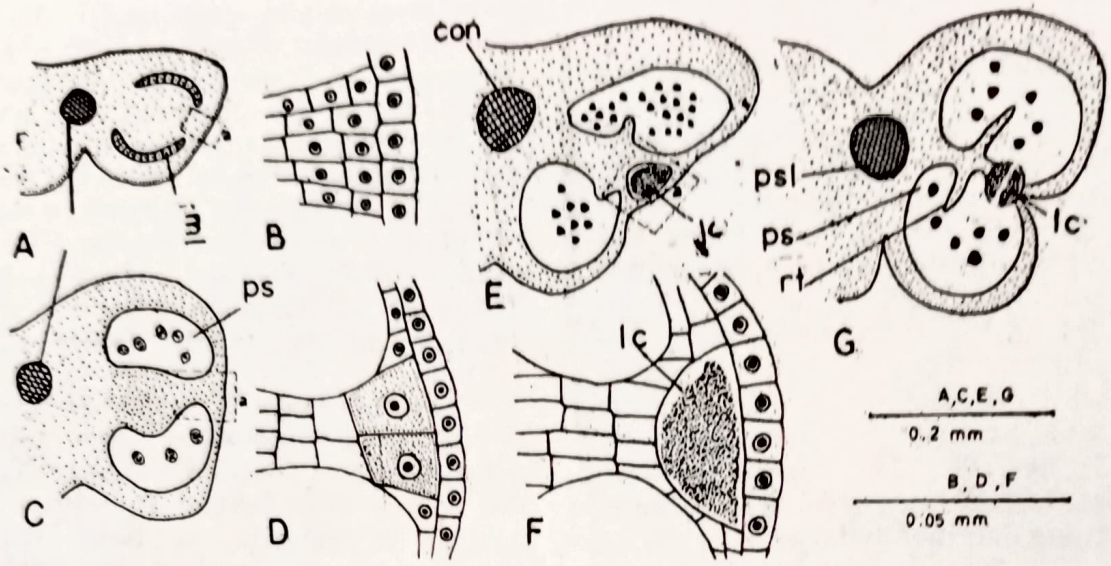
Text-figure 1

A. Cross section of a young anther.
B,C. Cross section of part of anther showing development of anther wall.
D. Same showing degenerating middle layers and tapetum.
E. Fibrous thickenings in the endothecial cells.
F,G. Microspore tetrads.
H. Uninucleate pollen grain.
I. Large, uninucleate quadrangular pollen grain.
J. Dyad.
K. Polyad.
L. Compound pollen.
M,N. Budding of pollen grains.
O-U. Cross section of anther showing formation of resorption tissue and lysigenous cavity.
V. Longitudinal section, part of ovule showing megaspore mother cell.
W. Same showing four megaspores arranged in a linear manner.
X. Same showing tetrads of megaspores; chalazal one is functional.
Y. Longitudinal part of ovule showing megaspore next to chalazal one is functional.
Z. 2-nucleate embryosac.
Z_1 4-nucleate embryosac.
Z_2 Organized female gemetophyte.
Z_3 Female gametophyte showing early organization of antipodal cells.
Z_4 Longitudinal section, part of integument at female gametophyte stage.



Text-figure 2

A-G. Formation of resorption tissue in cross section of anthers of different maturity. (Ant, antipodal cells, con, connective; ep, epidermis; en; endothecium; eg, egg; iep, inner epidermis; inti, integumentary initial; lc, lysigenous cavity, mmc, microspore mother cells; ml, middle layers; mgc, megaspore mother cells; m, megaspore; oep, outer epidermis; ps, pollen sac; m, megaspore; ppl, primary parietal layer; psl, primary sporogenous layer, rt, resorption tissue; sy, synergids; sn; secondary nucleus; t, tapetum).

forms lysigenous cavity in the septum between two anther lobes. Initially, the cells of the resorption tissue do not differ from the adjacent tissue of anther lobe. At the microspore mother cell stage, cells of the septum region between two pollen sacs are slightly elongated and there is no differentiation of hypodermal cells (Text-figs. 2A, B). At the tetrad stage, two to three hypodermal cells are well-differentiated and are larger than other adjoining cells (Text-figs. 2C, D). At the time of microspore formation these cells get converted into a lysigenous cavity which is densely filled with mucilaginous substance (Text-figs. 2E, F). At the time of dehiscence the resorption tissue is very much reduced, stomium cells separate and the anther wall splits through a longitudinal slit. At this stage the lysigenous cavity ruptures and its contents disintegrate (Text-fig. 2G).

Dehiscence of anther in the treatments is on the same pattern as control but in several cases of tetraploids treated with 50 k rad, rosorption tissue is reduced considerably.

Discussion

Tetrasporangiate condition of anther is of normal occurrence in control and many treatments, but in many cases of treatments anthers become five to seven-lobed. In a few cases, the number of lobes is reduced to two or three. The position of the anther lobes in an anther is found to be assymmetrical/displaced in many cases of treatments.

Many of the above mentioned variations have been reported earlier by Singh and Gunckel (1965). The increase or decrease in the number of anther lobes may be due to induction of excessive or reduced number of constrictions in the expanded portion of staminal primordia. Similar features have been observed by Chauhan (1969) in *Carthamus tinctorius*. Heslop-Harrison (1967) has suggested that floral primordia are sensitive to ionizing radiation and resulted to anther lobes variations.

Initial pattern of anther wall development in plants of control as well as treatments is identical. In general, the anther wall at the time of meiosis in microspore mother cells consists of a layer each of epidermis, endothecium, and one tapetum or two middle layers. In several cases of higher treatments in tetraploids all the wall layers except the epidermis degenerate. Such a condition has also been reported in *Cyamopsis tetragonoloba* by Rai (1971).

Endothecium is more distinct in the apical region of the anther lobe where its cells elongate radially and develop fibrous thickenings, but at other places it degenerates in all the ploidy groups. Saxena and Singh (1969) have also reported that endothecium degenerate in most part of mature anther. In plants of treatments also, the fibrous thickenings are found in the apical region of the anther, but in some cases of higher doses there is no formation of fibrous thickenings at all. Chauhan (1968) in *Ghenopodium album* has also not seen fibrous thickenings in the endothecium in many cases of treatments. Absence of fibrous thickenings in the endothecium makes the anthers non-dehiscent and this ultimately leads towards morphological male sterility.

Tapetum is glandular in nature and the tapetal cells lying towards connective are larger and more radially elongated as compared to those lying on the peripheral side. The tapetum, in general, degenerates by the time microspores are formed. In some cases of treatments the tapetum shows many abnormalities, e.g., early degeneration or persistence for longer period, excessive enlargement and sometimes intermixing with the microspore tetrads. Some of these abnormalities have also been observed by Singh and Mahana (1977) in *Physalis ixocarpa*. Variations in the structure and behaviour of tapetum in response to gamma irradiation have also been reported by Chauhan (1969) in *Chenopodium album* treated with chronic gamma rays.

Singh and Hadley (1961), Zenketler (1962), and Dubey and Singh (1965) have also reported persistent nature of tapetum in *Sorghum, Daucus* and *Linum* respectively. According to these workers the cause of microspore degeneration is the persistence of tapetum.

Variability in the arrangement of microspores in a tetrad has been seen during present investigation. Formation of dyads, triads and polyads is also observed in many cases. Formation of such structures has also been reported by several workers (Singh & Gunckel, 1965; Chauhan, 1968; Chopra & Singh, 1974). Four-nucleate and multinucleate pollen grains have been seen in several cases of treatments. Four-nucleate microspores may be due to failure of cytokinesis as also reported by Singh and Gunckel (1965) in *Ricinus communis*.

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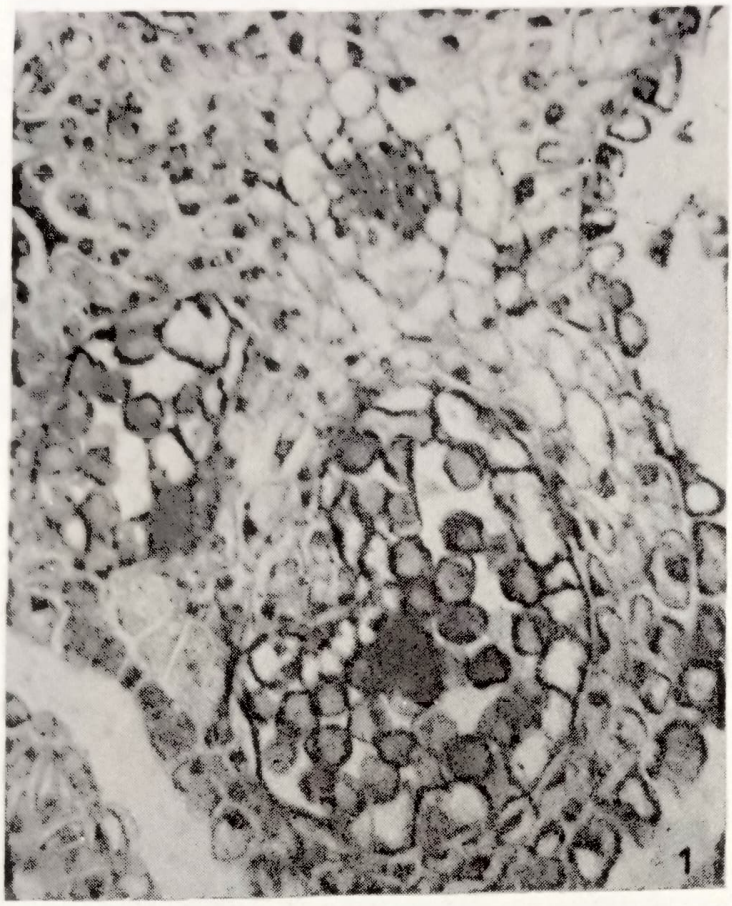
Explanation of Plate

Plate 1

1. Persistence of tapetum even at microspore stage (tetraploid 50k rad), x213.
2. One anther lobe showing degeneration of tapetum, while other shows its persistence (tetraploid 50k rad), x213.
3. Fusion of anthers in a cross section (tetraploid 50k rad), x73.
4. Unusual anthers in cross section (diploid 50k rad), x66.

Plate 2

1. Unusual anthers in cross section (diploid 50k rad), x66.
2. One anther is seen arising from petal (tetraploid 50k rad), x30.
3. Anther sterility in cross section (tetraploid 50k rad), x54.
4. 2 to 5-lobed anthers in cross section (diploid 40k rad), x66.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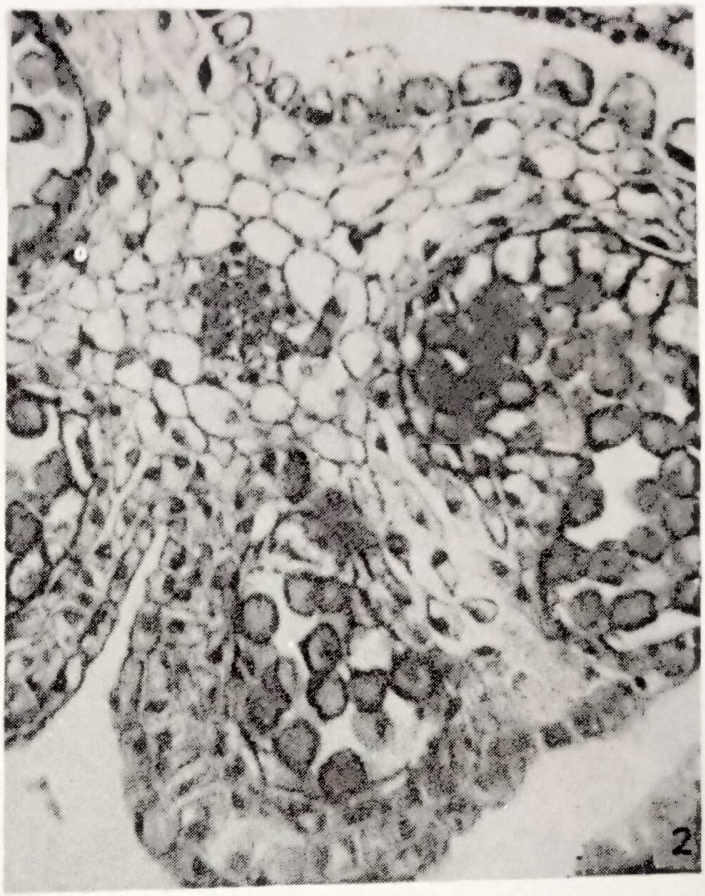
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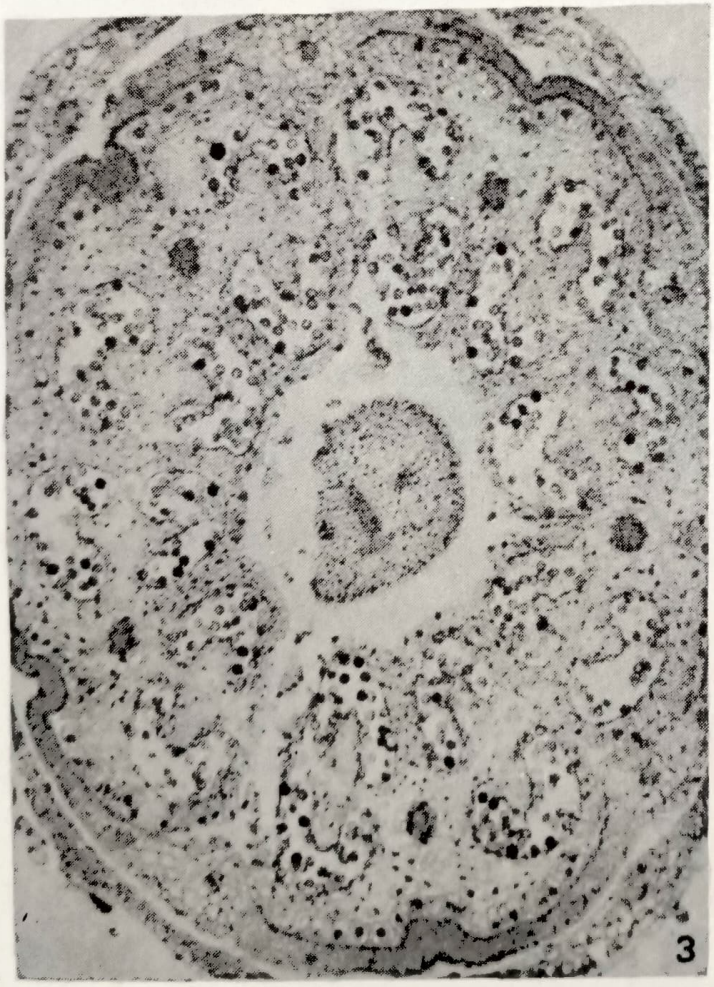
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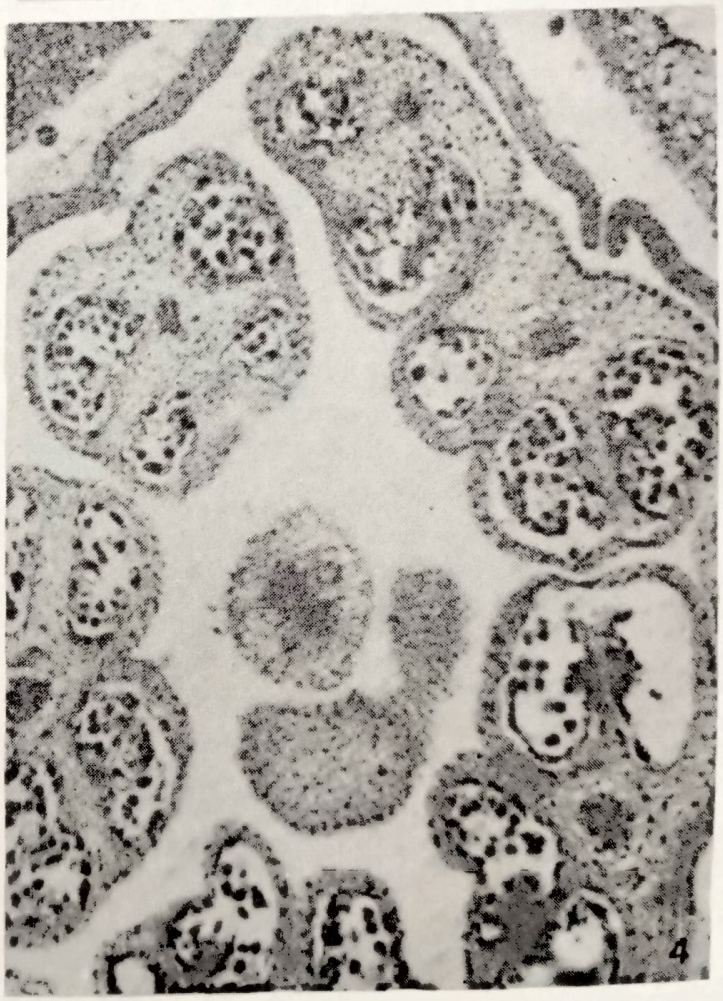
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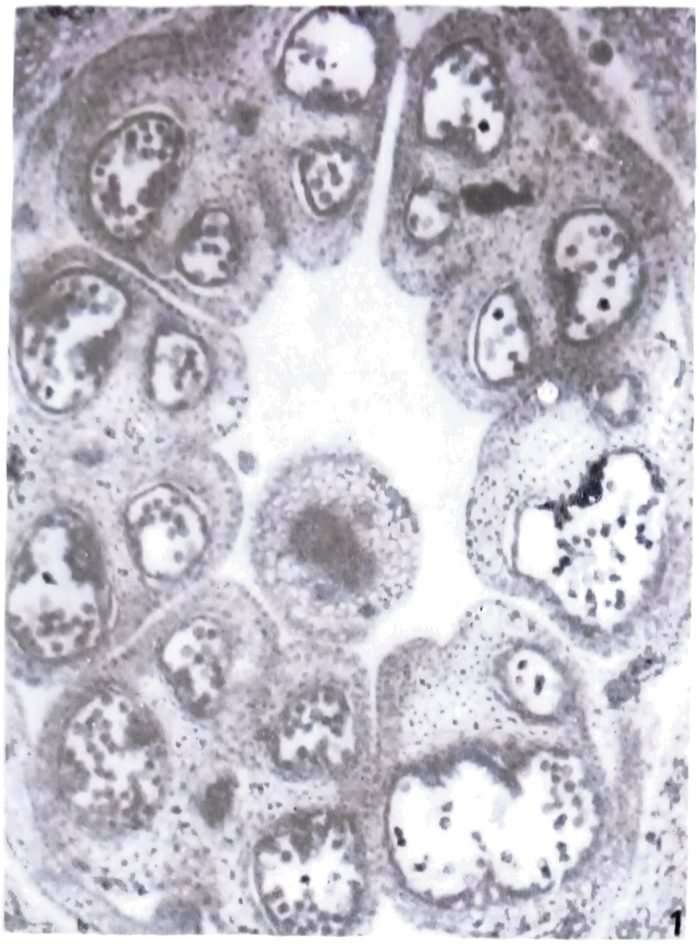

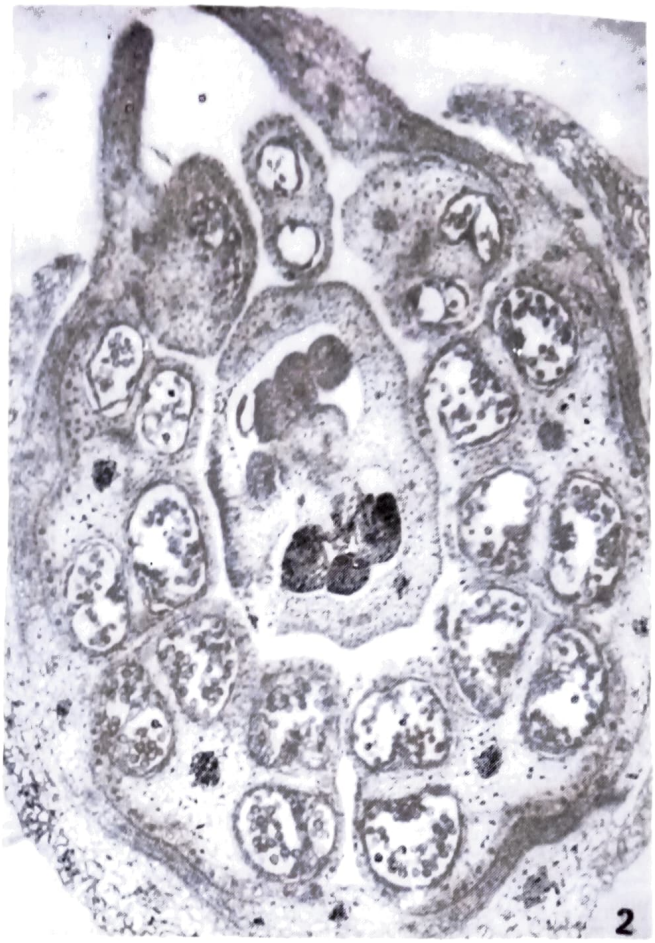

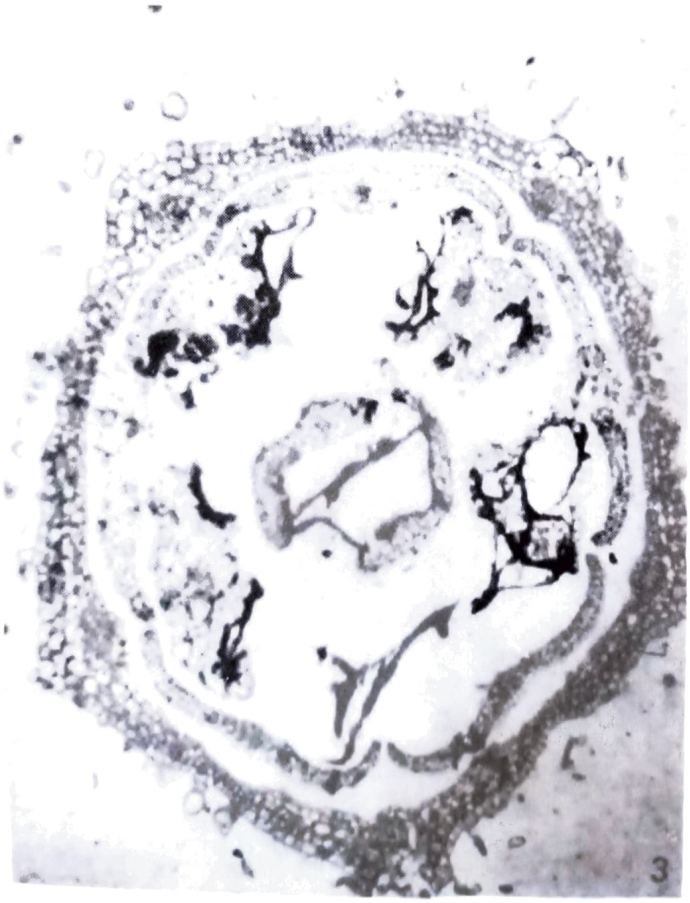

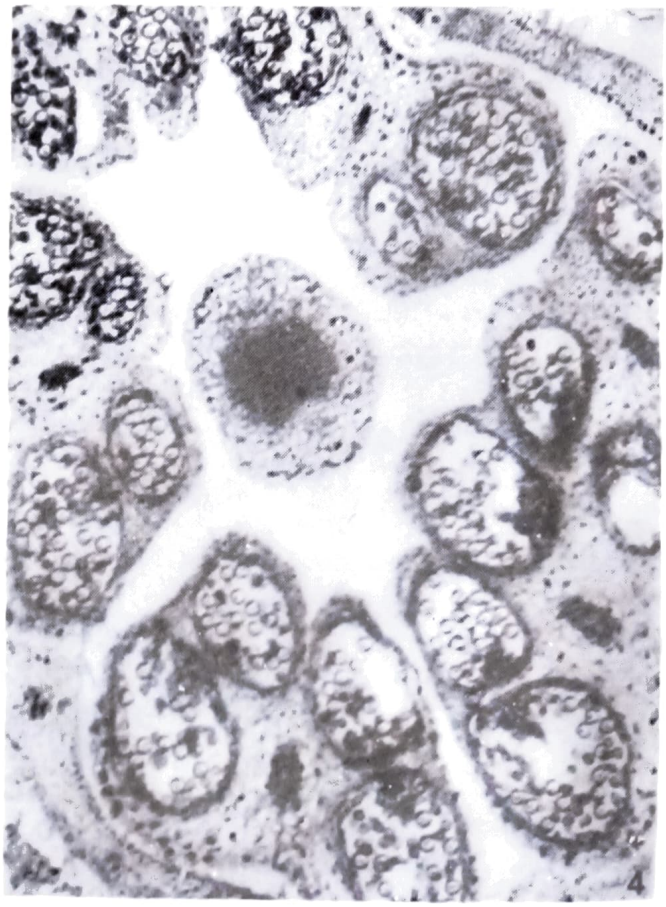

Chandel—**Plate 2**