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DEVELOPMENT OF OVULE IN YELLOW LUPINE UNDER CONDITIONS OF INFECTION WITH BYMV

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Transmission of virus infection from a diseased plant to its progeny may proceed through formation of infected seeds. This phenomenon substantially promotes spreading of infection. At present, 119 viruses transmitted by seeds of 162 plant species are known. Plant species differ in the percentage of virus-transmitting seeds. In only few cases, more than a half of seeds are infected [17]. Yellow lupine (Lupinus luteus L.) plants infected with bean yellow mosaic virus (BYMV) produce about 6.2% of infected seeds [7].

The present studies were designed to determine the location of BYMV particles at various developmental stages of yellow lupine ovule and to gain knowledge of the virus-induced changes, in order to establish which morphological elements of seed are responsible for virus transmission.

METHODS

The plant material was derived from two greenhouse experiments. In the first one, yellow lupine (Lupinus luteus L.) plants were sown on 5 September 1983, and in the second - on 6 February 1984. Plants were inoculated with BYMV on 29 September 1983 and 13 March 1984, respectively. Mechanical inoculation was performed in the phase of leaf rosette, i.e. at the stage of 3-4 developed leaves. The three youngest, well developed leaves were inoculated.

At about 2 months after inoculation, from every series four samples each of the BYMV-infected and healthy plants were collected at 7-day intervals. At this time, the plants were in the phase of a big flower bud, florescence and slight flower-shedding, respectively. A part of plants formed side shoots with visible germs of inflorescences.

Fragments of the vegetative of plants (stems and petioles) were collected. From the generative parts, in dependence on the developmental stage either the whole flower buds or the pistils alone were isolated. The material for inspection under a light microscope was fixed in FAA (mixture of ethanol, formalin ang glacial acetic acid 90:5:5). It was dehydrated with graded concentrations of ethanol and xylene, and then embedded in paraffin. It was cut in a Reichert rotary microtome. After removal of paraffin with xylene, sections were stained with iron-alum hematoxylin according to Heidenhaim. A Carl Zeiss Jena NU2 light microscope was used.

The material for ultrastructural studies was fixed in Karnovsky fixative (mixture of 3% of glutaraldehyde and 4% of paraformaldehyde in cacodylate buffer pH 7.2) for 2 h at room temperature. Additionally, the material was fixed in 1% OsO_4 for 2 h at 4°C. It was dehydrated with graded concentrations of ethanol, acetone and propylene oxide, whereupon it was embedded in Epon 812. It was cut in a LKB ultramicrotome. Sections were stained with lead citrate and uranyl acetate. Observations were taken in a JEM 100C electron microscope.

RESULTS

Yellow lupine plants inoculated with BYMV exhibited the first symptoms of systemic reaction 2 1/2 weeks after inoculation. The youngest leaves showed vein clearing combined with slight mottle and fine mosaic. A part of young leaves displayed rolling along the central vein and underdevelopment of individual leaflets.

In the course of plant growth, leaf blade mosaic became more intense and dark-green insular spots appeared. The leaflets of the youngest leaves were narrowed and the angle between leaf blades and petioles was changed. The BYMV-inoculated plants tended to form many lateral stems. The major part of flowers of the infected plants were underdeveloped (Fig. 1). They had distorted corolla petals. The carina-forming petals failed to be fused. Most flowers fell off prior

to pod setting. The few pods set were irregularly situated in the whorls. Young flower buds were tightly enveloped in hairy leaflets. The cells of these leaflets, mostly of a mesophyll nature, contained many viruses and their companion structures.

BYMV belongs to the Potyvirus group. It is a rod-shaped virus, 680--690 nm in length and 11 nm in diameter. BYMV particles always occurred in the cytoplasm of the infected cells either in regular aggregates or loosely scattered (Figs 8, 9). They were accompanied by "pinwheel"-type structures. The structures accompanying BYMV infection also comprised elongated cisternae of different diameters, surrounded by a single membrane and filled with an electron-opaque homogeneous substance. They usually occurred in the vicinity of microbodies resembling peroxisomes and were filled with a very fine fibrillar material. They were accompanied by virus particles. The protoplast of these cells was very poor, being almost completely devoid of plasmatic membranes and organellae.

The development of flower buds, and particularly of their generative elements, was very differentiated. If not shed very early, the buds developed flowers whose pistils were degraded to a differed extent. The first visible symptom consisted of blasting of pistils in their apical part; subsequently, this process involved the whole ovary. There was narrowing of the ovular chamber in which one or two distorted ovules occurred. These ovules exhibited no differentiation into nucellus and integuments. No ovular sacs were formed in them. The ovule consisted of a mass of necrotized cells with homogeneous protoplasts. Such pistils, together with ovules, blasted and failed to produce pods and seeds (Fig. 2 a). In some pistils the pathologic process proceeded less intensely. In the ovules the initial stages of macrosporogenesis, inclusive of the stage of tetranuclear ovular sac, took place. The ovular sacs were distorted. The nuclei, together with a small amount of protoplast, were concentrated in the central part of the sac (Fig. 2 b, c).

The pistils situated in the apical part of the inflorescence showed the least changes. Their shape was elongated, as typical of lupine. In the ovary, usually 4-6 anatropous ovules were formed. They were surrounded by two integuments. The external, thicker integument comprised several layers of cells, whereas the internal one was two--layered, with regular and compactly arranged cells. On the side of the ovary lumen, they comprised cutinized cell walls and big vacuoles.

The tenuinucellar nucellus contained cells of a meristem nature; they were compactly arranged and had rich protoplasts with big cell nuclei. In the chalazal part, the end of a vacular bundle was observed. The parental cell of the macrospore appeared the micropylar part of the ovule. It was ova-shaped and had callose thickenings of the cell wall. It seams that the course macrosporogenesis was undisturbed. There were 1-, 2-, 4- and 8-nuclear ovular sacs. The antipodal nuclei became disintegrated relatively rapidly. Figs 3-7 show fragments of ovular sacs at various developmental stages, together with the surrounding cells of the nucellus.

In no case were the BYMV particles and accompanying structures present in nucellus cells and archesporial cell, or at any developmental stage of the ovular sac. BYMV particles, pinwheel structures and electron-opaque cisternae occurred in big numbers in all vegetative parts of the infected plants. They were found in the ovary wall. The external and internal integuments of the ovule were the last cell zone containing BYMV particles.

The presence of viruses in the integuments caused no visible changes in the morphology of ovules and their development. Therefore, these ovules could - after fertilization - produce seeds transmitting BYMV particles in the seed coat. In infected cells occurring in various parts of the plant, very many osmophilic deposits were present. This substance was of the nature of either dispersed structures accumulated in the vacuole or distinct globules associated with plasmalemma or with the tonoplast (Fig. 10 a-c). It seems that their presence was due to degradation of the protoplast, and particularly of that of cytomembranes.

DISCUSSION

Viruses can be transmitted by different parts of seed. In seed, they may remain in the state of rest, be activated or inactivated [1, 10, 14]. They nearly always cause losses of seed crop. In BYMV-infected pea, Błaszczak and Zdybek-Szyld [3] have found a drop in the number of seeds by 32-43% and a drop in weight of seeds by 46-54%. Mandahar [17] has presented a number of factors influecing the extent of seed infection; they include genetic determination, susceptibility degree of cultivars of the given species, virulency of various virus strains, time elapsed from infection, and external factors (e.g. temperature).

Viruses can occur on the external surface of seed coat or may penetrate into its internal ports. Crowley [8] has observed in seed coat cells of bean the common mosaic virus (BCMV), tomato spotted wilt virus (ToSWV) and cucumber mosaic virus (CMV). Fiedorow [9] has reported for BYMV-infected broad bean plants that only 0.2% of seeds afforded diseased seedlings which - in turn - yielded about 11% of infected seeds. Using test plants, Fiedorow has detected the virus in corolla petals, stamens, pistils and partly in pollen; the virus was present in 0-11% of mature seeds. It was most often (in 85% of seeds) isolated from the seed coat.

Seed infectivity may be associated with infection of the young, progeny sporophyte already at the embryonic stage. According to the literature data, the infection of the embryo can be induced by an infected pollen grain, invasion of a meristem, ovular sac or the embryo itself. Paccini and Cresti [18] have suggested that viruses can be eliminated from the maturing microspores and enclosed in germinative pores by deposits of pectin-cellulose substances. Hamilton et al. [12] have observed TMV and BMV particles on the surface of exine; protoplasts of microspores were free from viruses. Likewise, Garbaczewska and Golinowski [11] have found no BYMV particles in developing or mature grains of yellow lupine pollen; on the other hand, virus particles were observed in the tapetum and other tissues of the yellow lupine parental plant. Carroll and Mayhew [6] have demonstrated the presence of BSMV particles (seed-transmitted strain) et all developmental stages of microspores as well as in cytoplasm and nuclei of sperm cells. These authors have detected bean Southern mosaic virus (BSMV) particles also in integuments and nucellus of developing barley ovules; they were present in the parental cell of the macrospore, in the macrospore and in all developmental stages of the ovular sac and of the embryo itself. BSMV particles have been found to be connected with microtubules of the karyokinetic spindle and with the cytoplasmic microtubules. These authors suggest that - - likewise - the apical meristem is not protected against infection with BSMV. However, it is generally assumed that viruses are unable to penetrate into apical meristems. Bennet [2] is of the opinion that virus infection can take place only in the very early developmental stages of vegetative meristems; the lack of plasmodesmal links between the embryo and the parental plant prevents virus infection of the former. Moreover, as suggested by Schippers [19], disintegra-

tion of the nucellus cells around the developing ovular sac protects the zygote and embryo against the infection. Ie reports the presence of TMSV in - only - the somatic tissues of the ovule, whereas the ovular sac and the surrounding cells are free from viruses. Kazimierski and Kazimierska [15, 16] described in BYMV-infected yellow lupine plants underdevelopment of ovules and distortion of ovular sacs (without the presence of virus particles in the ovule). The present observations also testify to developmental disturbances of the generative parts, e.g. to an abnormal structure of the flower corolla, or degeneration of pistils. BYMV particles spread - as a result of systemic infection - to all tissues of the parental plant. They are present in ovary wall cells and in ovular integuments. Therefore, they may occur also in the seed coat. According to the present results, neither BYMV particles nor cytopathologic structures are found in various developmental stages of the ovular sac. It can thus be assumed that the applied BYMV isolate encounters considerable barriers during penetration into the female gametophyte of yellow lupine.

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ROZWÓJ ZALĄŻKA ŁUBINU ŻÓŁTEGO W WARUNKACH INFEKCJI ROŚLIN WIRUSEM BYMV

S t r e s z c z e n i e

Przeprowadzono badania mikroskopowe roślin łubinu żółtego porażonego wirusem żółtej mozaiki fasoli (BYMV). Cząstki wirusa stwier-

dzono w wegetatywnych częściach porażonych roślin oraz w osłonkach zalążka. Wolne od wirusa i struktur cytopatologicznych były komórki ośrodka zalążka, makrospora i woreczek zalążkowy. Wydaje się, że opisywane w literaturze porażenie nasion przez BYMV związane jest z przenoszeniem wirusa w łupinie nasiennej.

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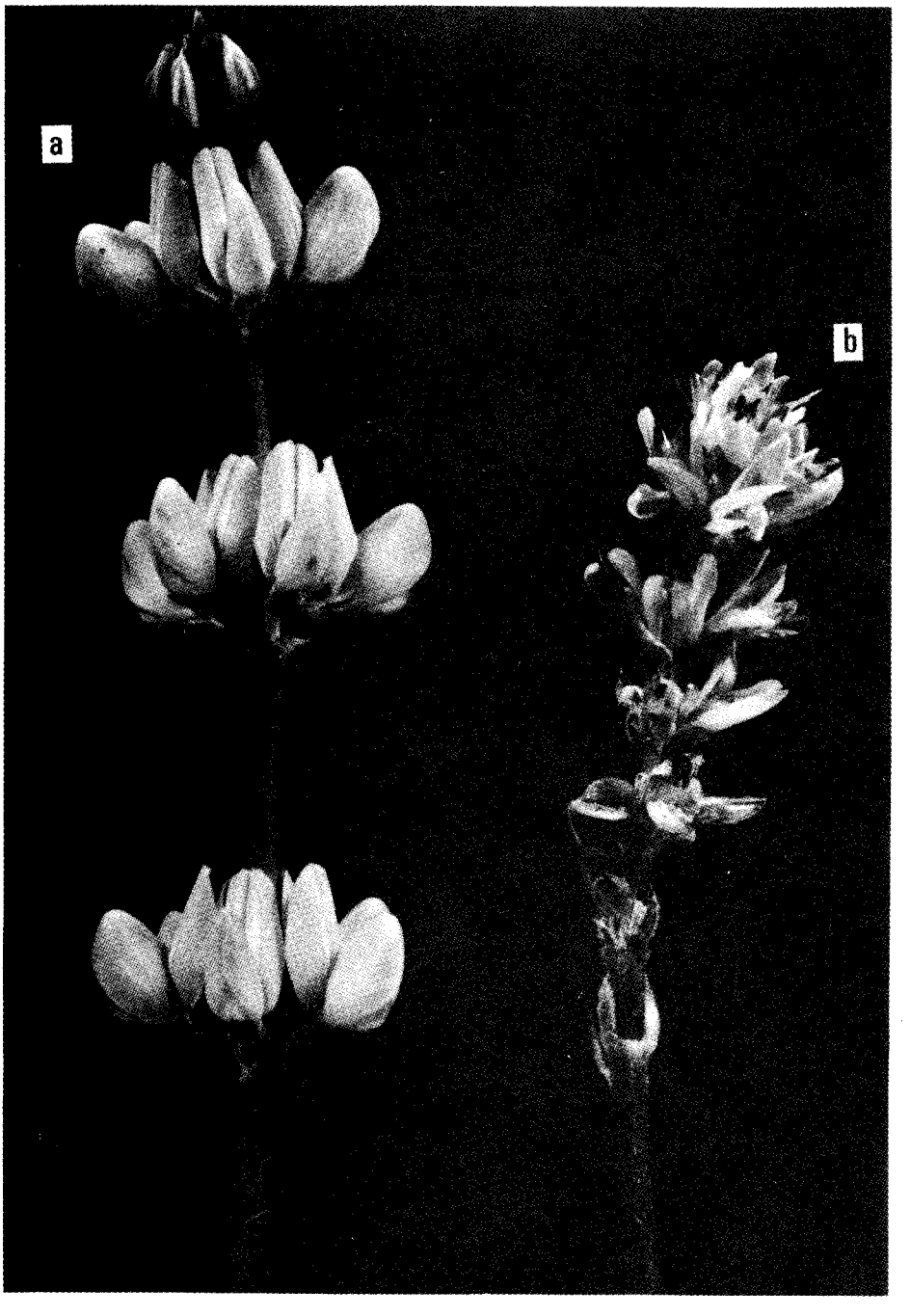
РАЗВИТИЕ СЕМЯПОЧКИ ЖЁЛТОГО ЛЮПИНА В УСЛОВИЯХ ИНФЕКЦИИ РАСТЕНИЙ ВИРУСОМ BYMV

Р е з ю м е

Проведены были микроскопные исследования растений жёлтого люпина, поражённого вирусом жёлтой мозаики фасоли (BYMV). Следы вируса были обнаружены в вегетативных частях и в оболочках семяпочки поражённых растений. Чистыми от вируса и цитопатологических структур оказались клетки ядра семяпочки, макроспора и зародышевый мешочек. По всей вероятности обсуждаемое в литературе поражение семян вирусом BYMV связано с переношением вируса в кожуре семени.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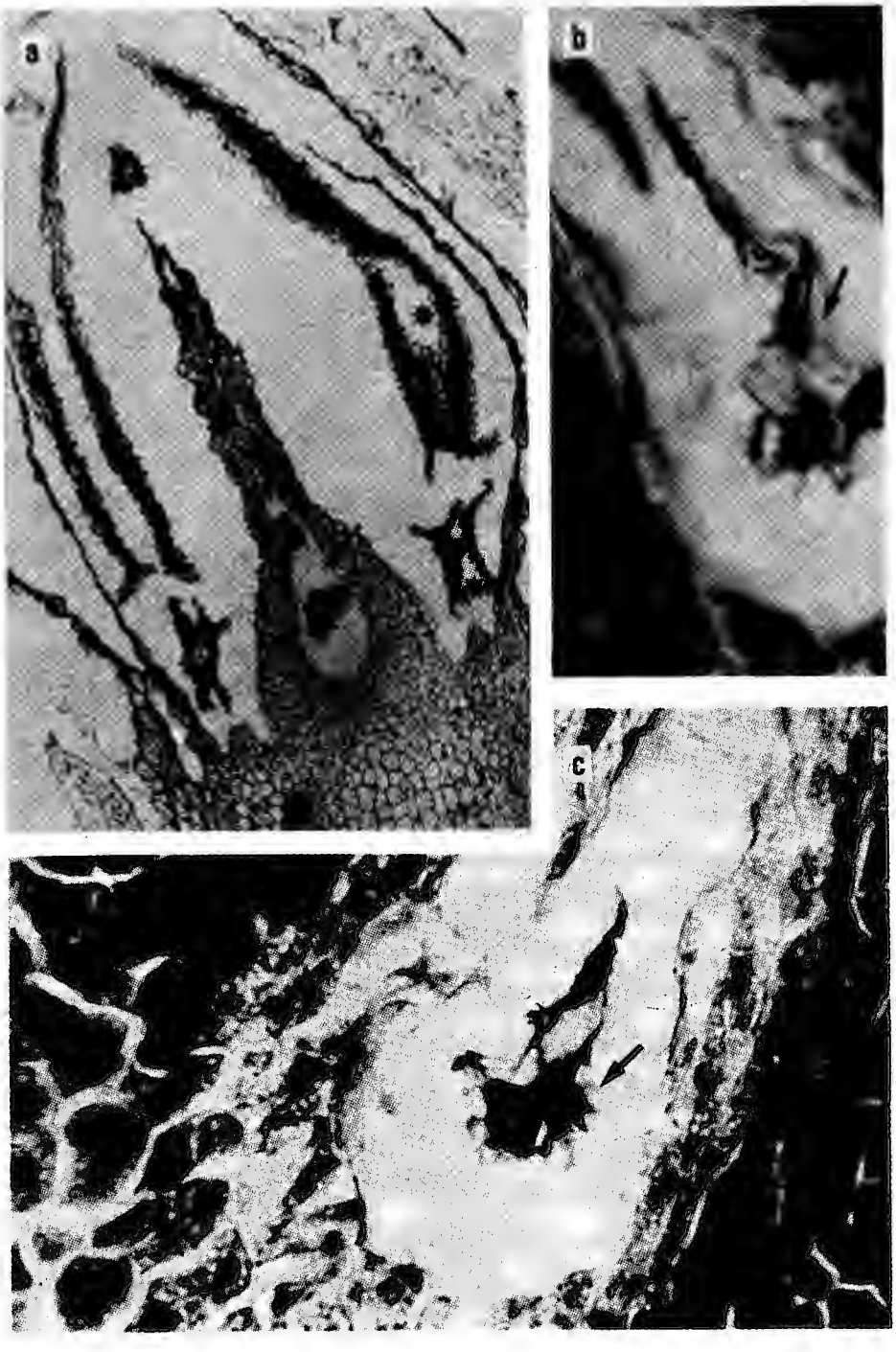
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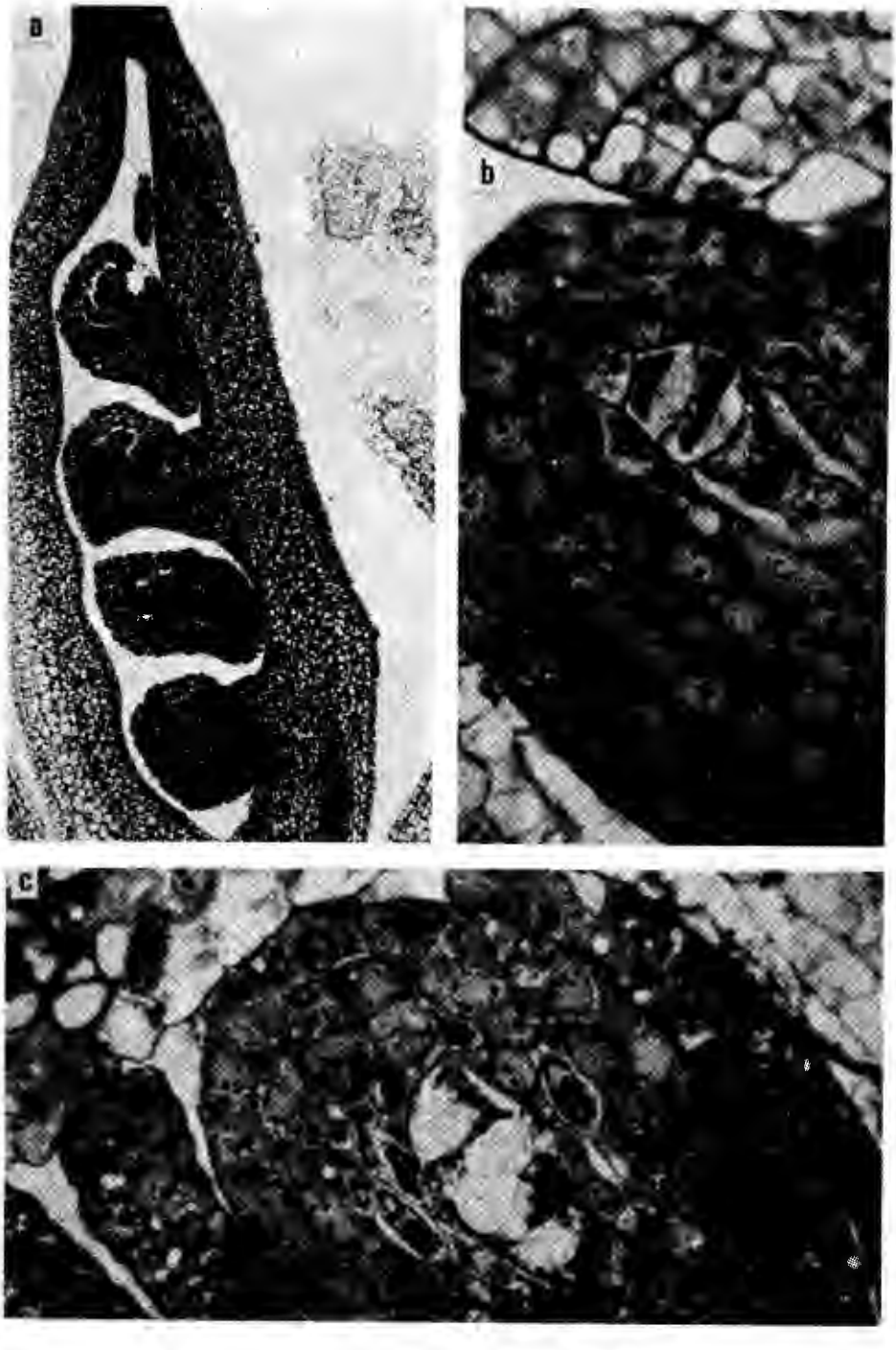
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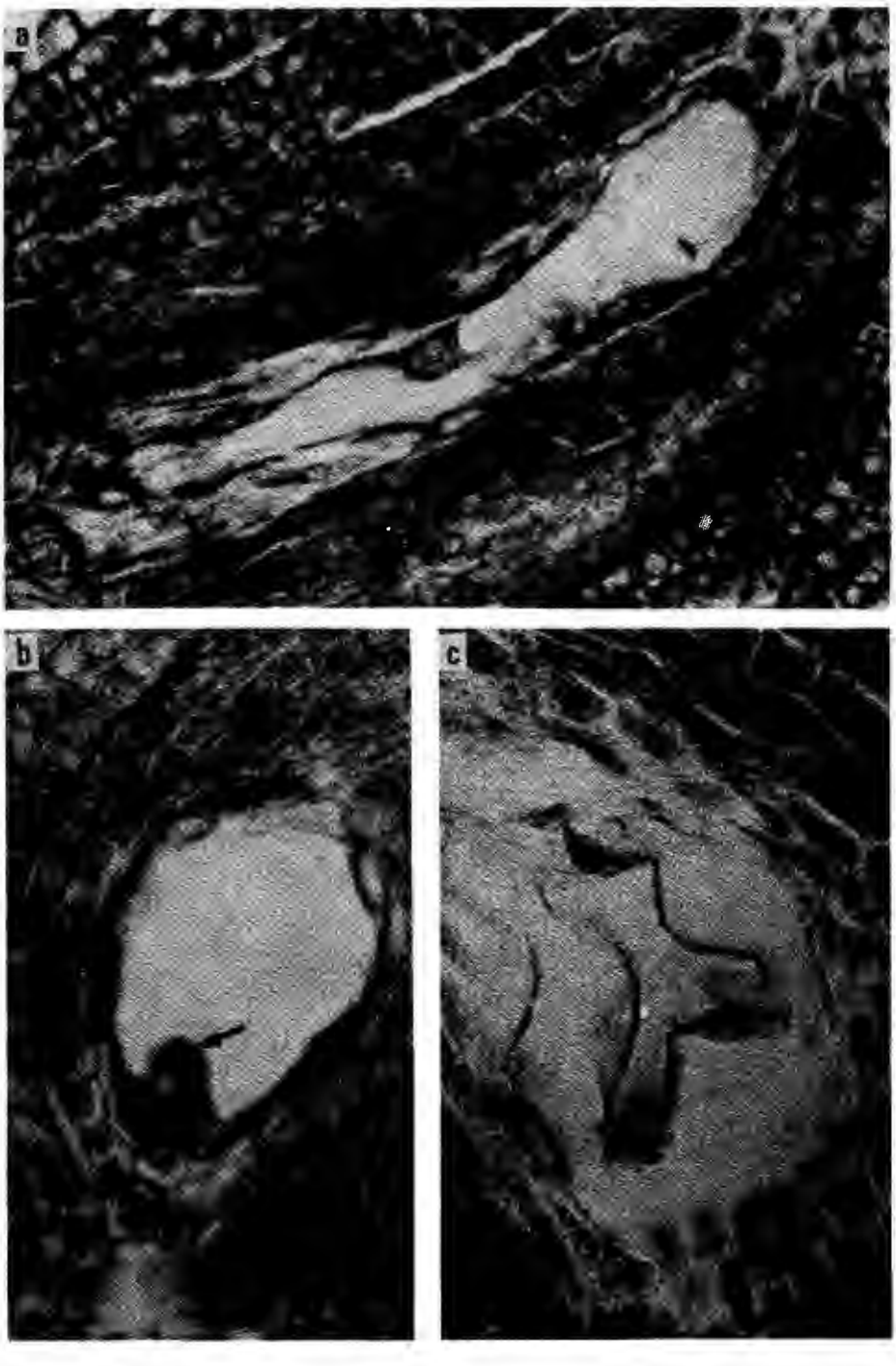
Fig. 1. Inflorescences of yellow lupine; a - control plant, b - BYMV--infected plant - magn. 0.3 x

Fig. 2. BYMV-infected plant; a - blasting flower bud. Visible necrotized stamens (asterisks). The pistil distorted in the upper part. In the ovular chamber - two distorted ovules (arrows) - magn. 120 x; b and c - necrotized tetranuclear ovular sacs (arrows) - magn. 750 x

Fig. 3. Pistils and ovules of flowers situated in the apical parts of inflorescences, in BYMV-infected plants; a - ovary with ovules - magn. 120 x; b - post-meiotic tetrad of cells in nucellus in ovule. Visible three disappearing cells near the micropyle and enlarging ovular sac (arrows) - magn. 750 x; c - uninuclear ovular sac (arrow) - magn. 750 x. On Figs b and c - visible the unchanged structure of ovule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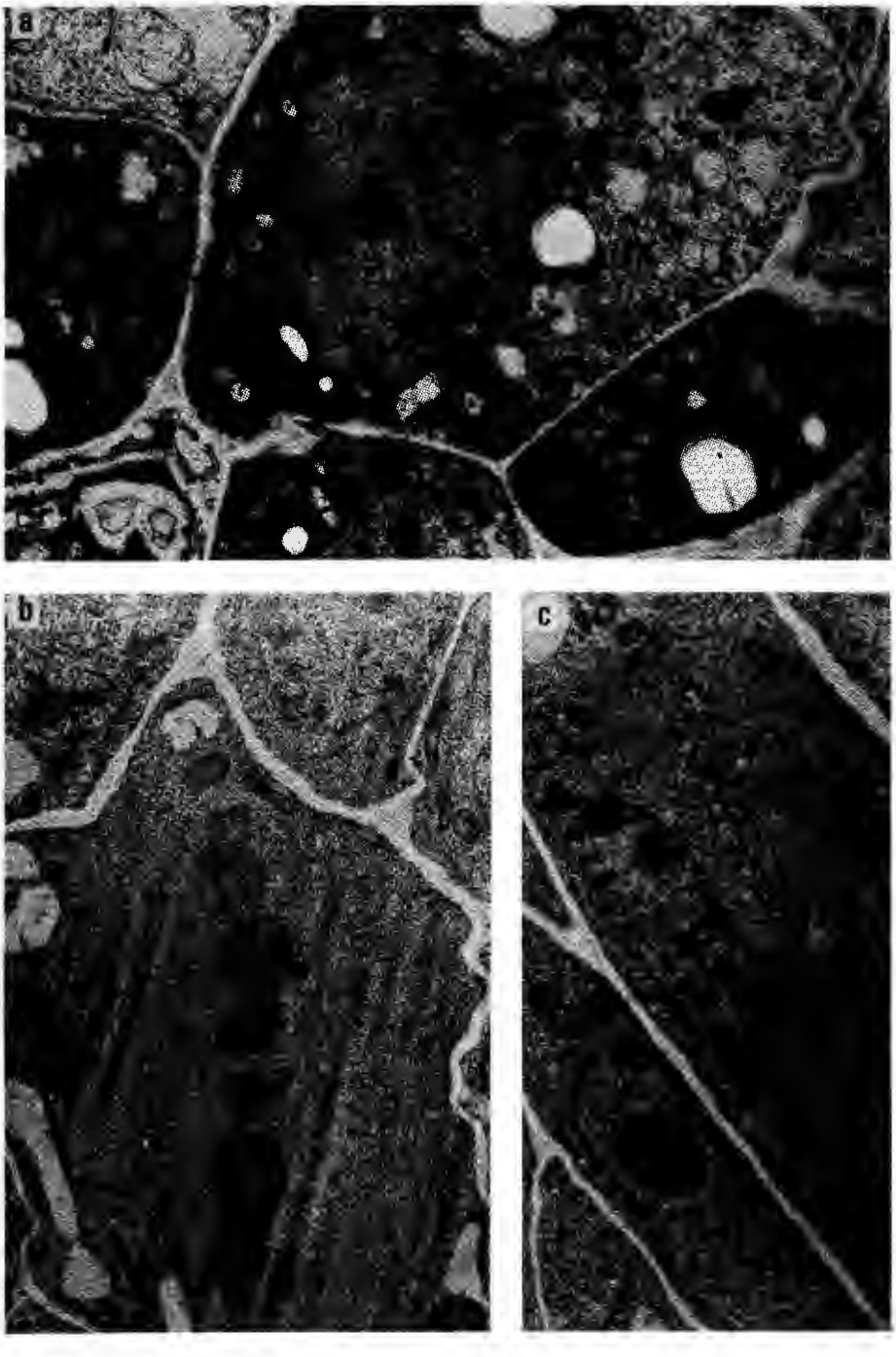
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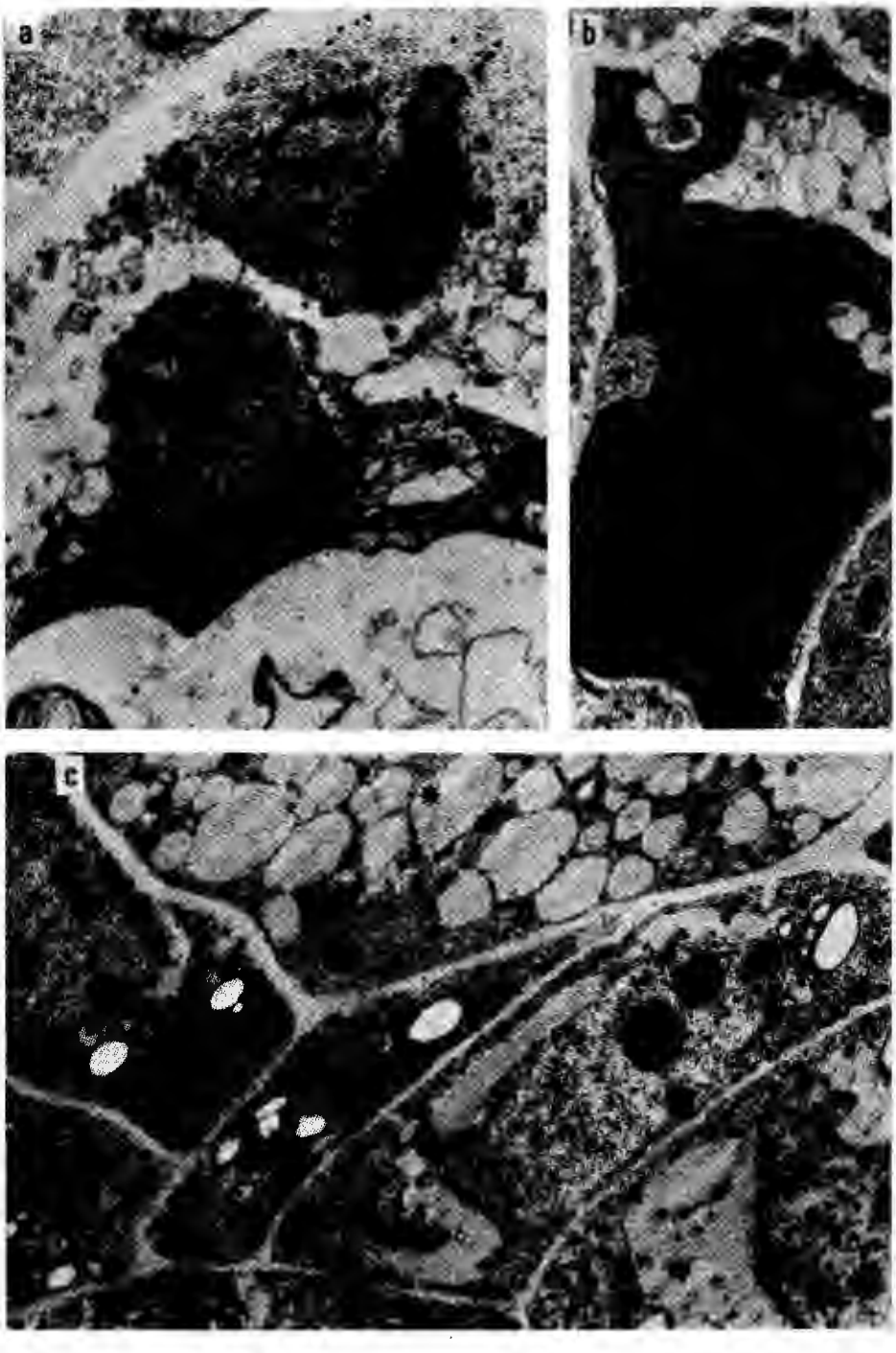
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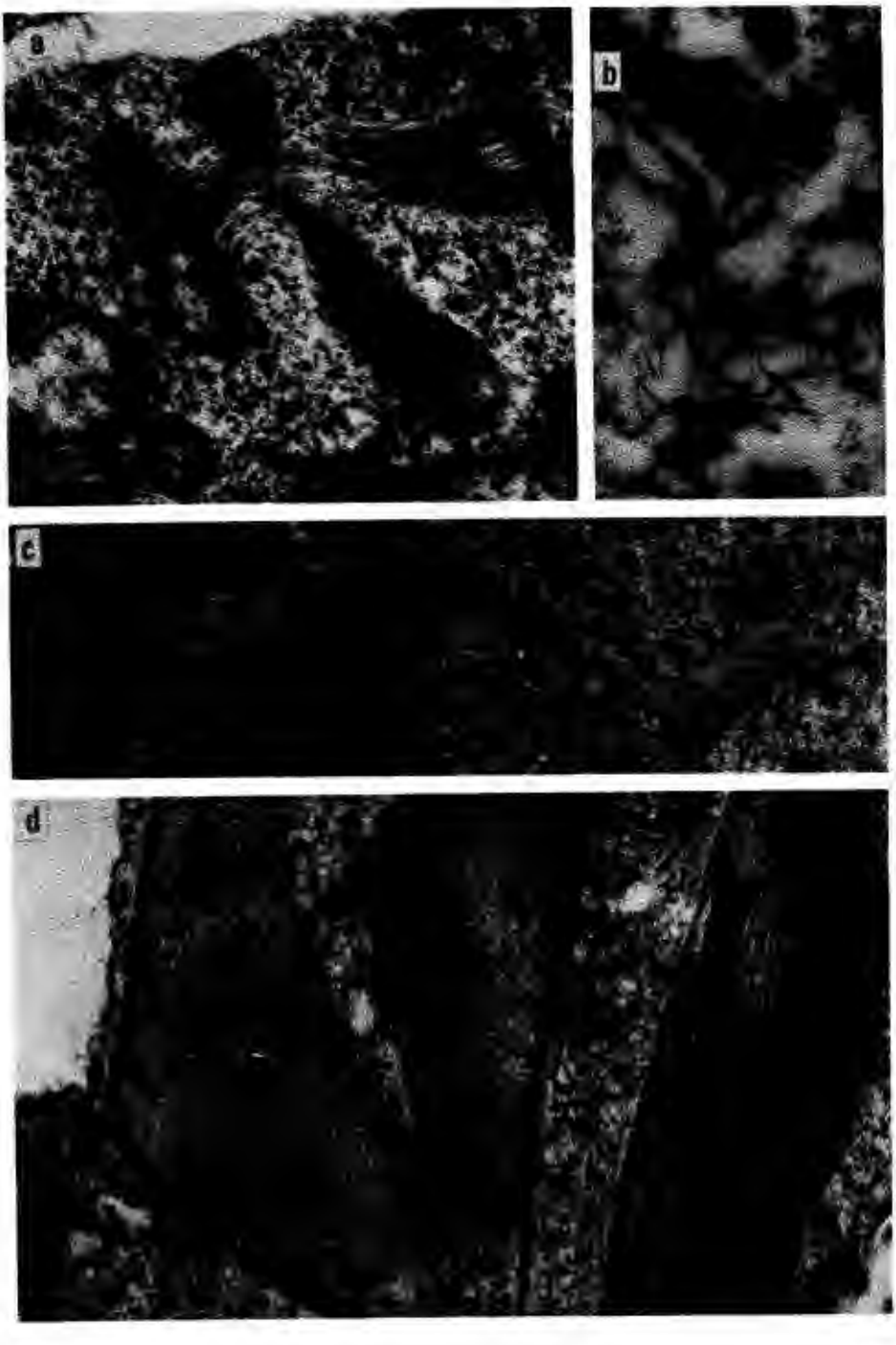
Fig. 4. BYMV-infected plants; a - mature ovular sac. In the centre - - visible a secondary nucleus (arrow) - magn. 750 x; b - egg apparatus of mature ovular sac. Egg cell (arrow) and two synergids situated nearby - magn. 1200 x; c - antipodal nuclei of ovular sac - magn. 1200 x. In all photographs, visible changes (characteristic of the given developmental stage of ovule) in nucellus cells surrounding the ovular sac.

Fig. 5. Development of ovular sac in BYMV-infected plants; a - fragment of parental cell of macrospore. Visible callose thickenings of cell wall (arrows) - magn. 5000 x; b - macrospore in the metaphase of first nuclear division - magn. 4800 x; c - binuclear ovular sac - magn. 7500 x. In all photographs visible unchanged nucellus cells. No viruses

Fig. 6. Development of ovular sac in BYMV-infected plants; a and b - - fragments of tetranuclear ovular sac - magn. 15000 x. Visible vacuolization of protoplast; c - fragment of octonuclear ovular sac (asterisk), with adhering unchanged nucellus cells. No viruses; magn. 7500 x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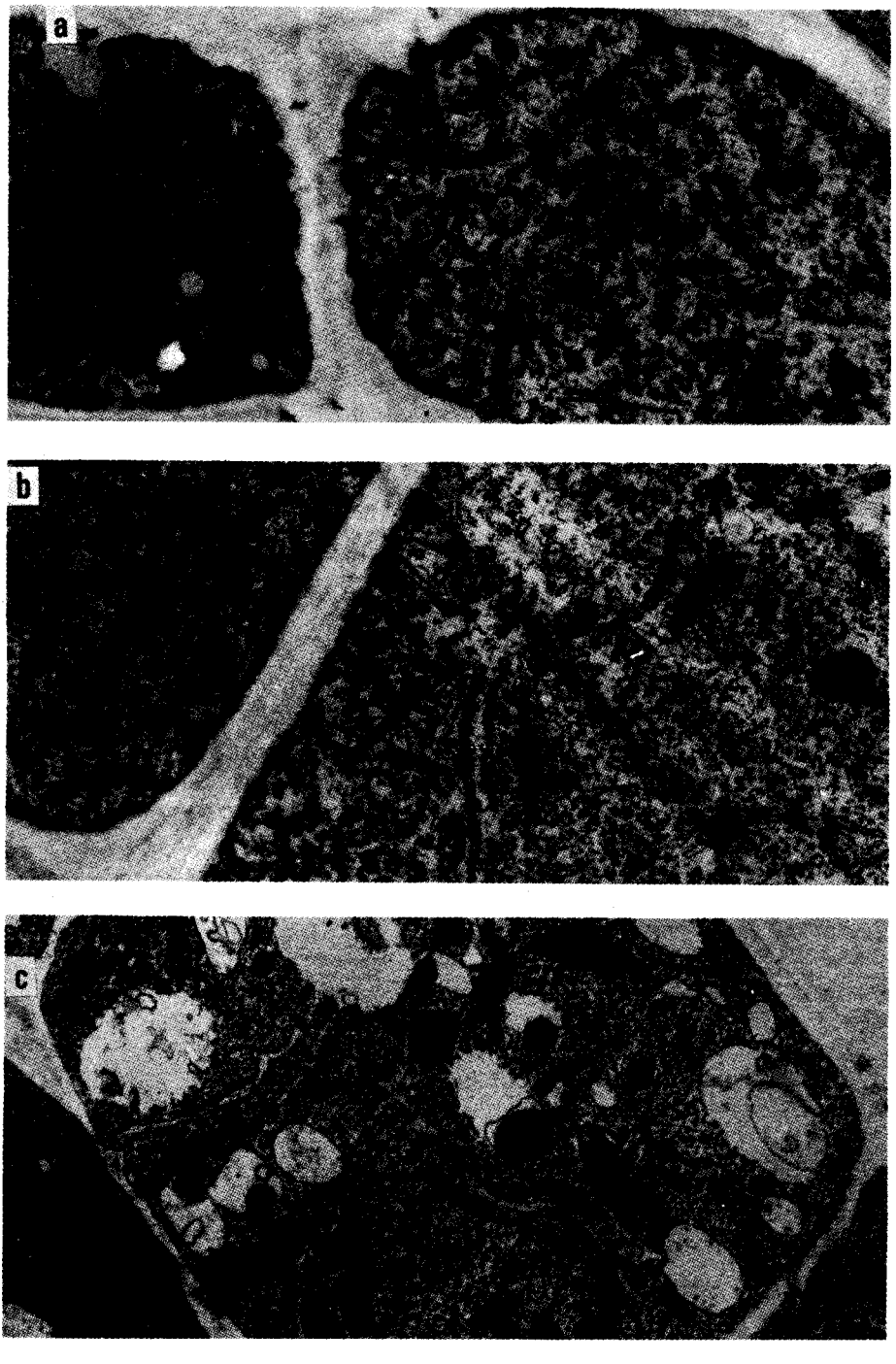
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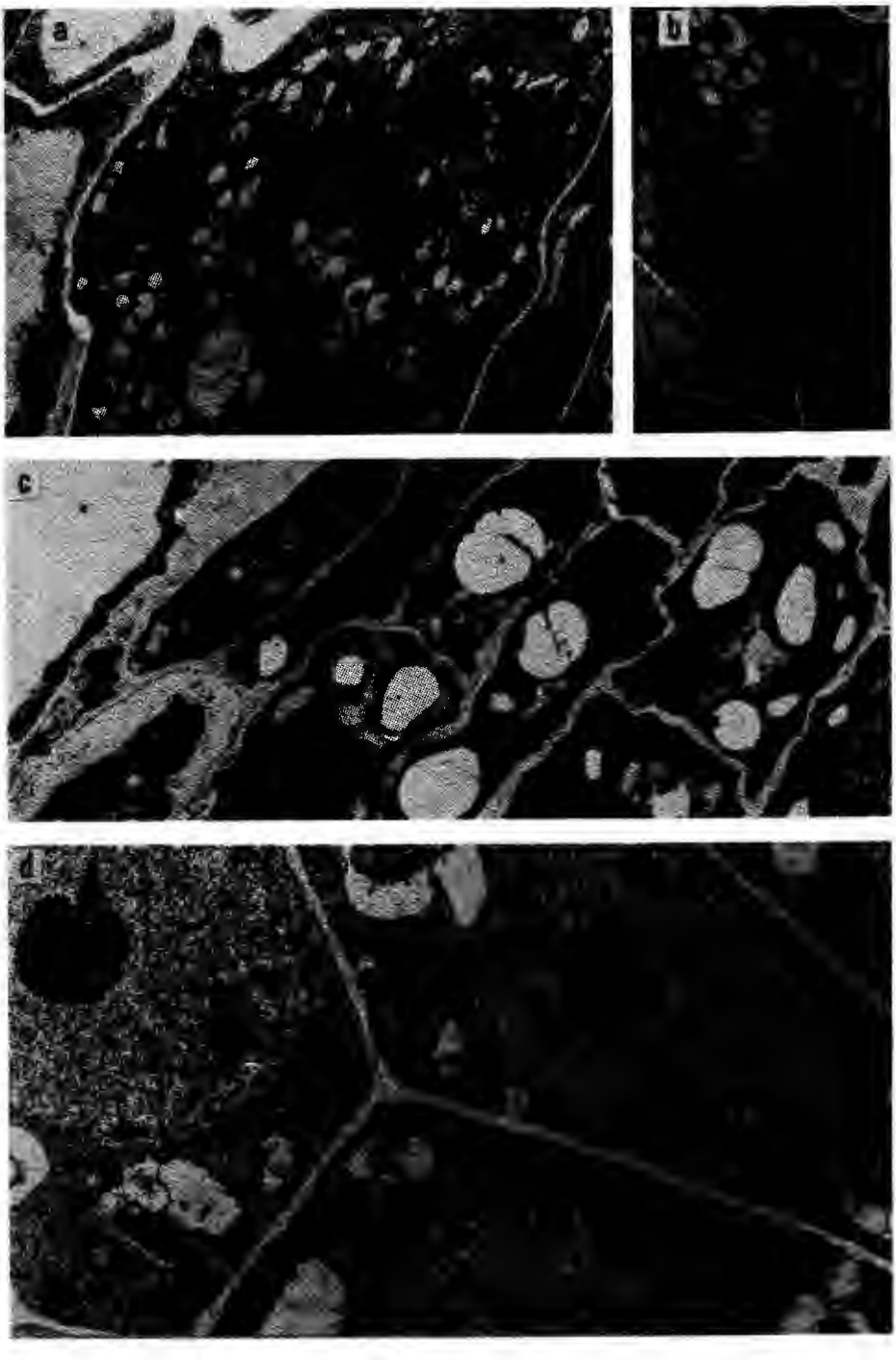
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Fig. 7. Development of ovular sac in BYMV-infected plants; a and b - - fragments of mature ovular sac - magn. 7500 x; c and d - visible nucellus cells. No viruses - magn. c 4800 x, d 1500 x

Fig. 8, Fragments of ovular integument; a - virus-infected cells of internal ovular integument - magn. 15000 x; b - virus-infected cells of external ovular integument - magn. 15000 x; c - cells of epidermis coating the wall of ovular chamber. Visible viruses and accompanying structures of pinwheel-type. No changes in protoplasts, caused by the presence of BYMV particles (arrows) - magn. 7500 x

Fig. 9. Cytopathology of BYMV; a,b,c,d - fragments of mesophyll cells of leaf, leaflet enveloping the flower bud and ovary wall. Everywhere visible BYMV particles (arrows) and long electron-opaque cisternae situated around structures resemblin peroxisomes (asterisks) - magn. a, b,c 30 000 x, d 50 000 x

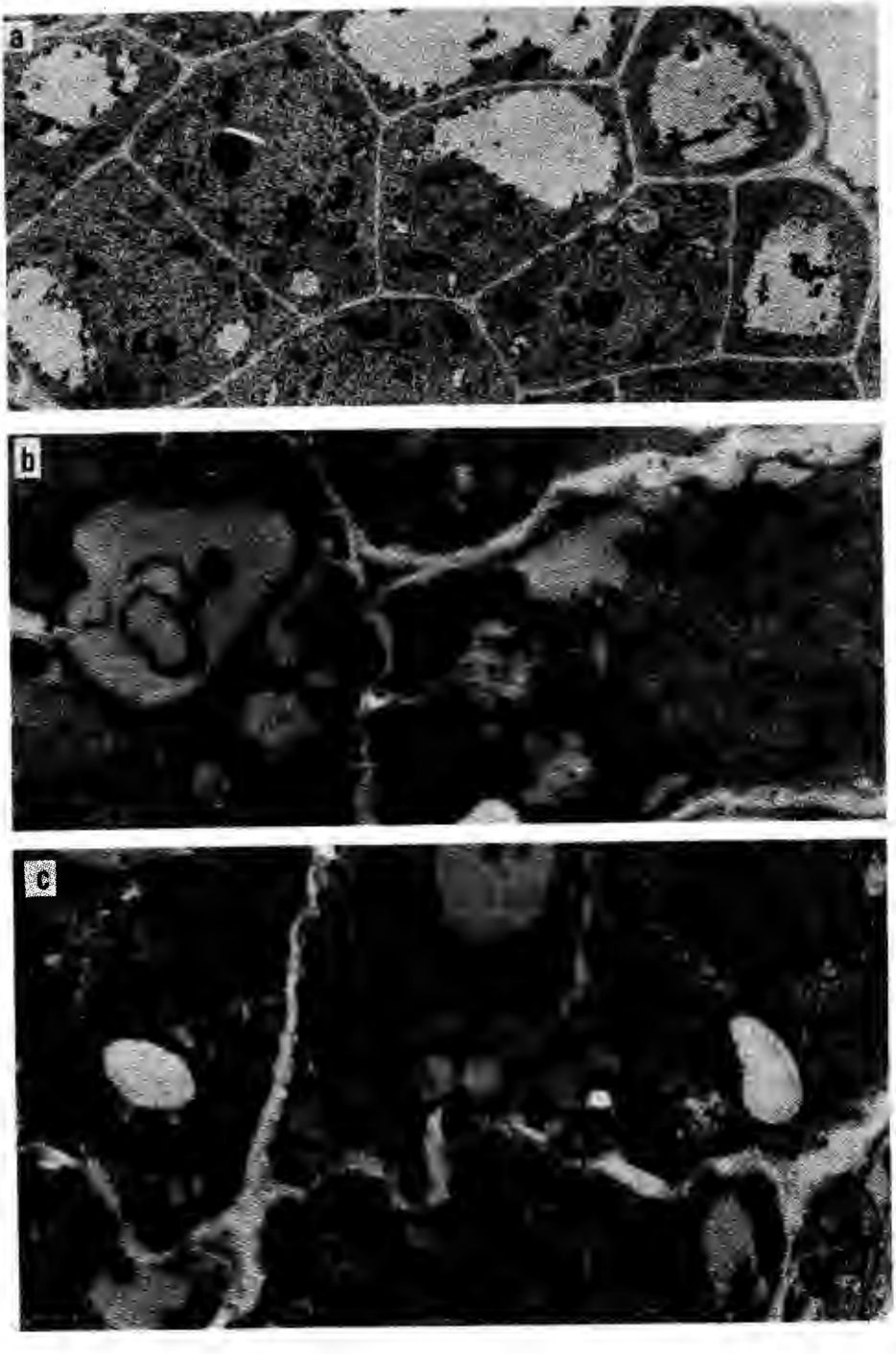
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Fig. 10. Fragments of ovular cells derived from the chalazal part; a - in vacuoles of these cells visible many fine osmophilic substances (arrows) - magn. 4800 x; b and c - similar substances located near plasmalemma of mesophyll cells of ovary wall - magn. 15 000 x