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Disturbances in the development of the female gametophyte in fully fertile tomatoes (*Lycopersicon esculentum* L. Mill) and those showing a tendency to parthenocarpy

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Abstract

It was found that part of the ovules in two lines of *Lycopersicon esculentum:* Kholodostoykye (Kh, fertile) and A33 (with a tendency to parthenocarpy) show disturbances in the development of the embryo sac. These irregularities can be seen in four phases: pre-meiotic, post-meiotic (tetrad), nucleate and cellular. The majority of irregularities were observed in the cellular stage of embryo sac development. The total number of ovules with disturbed female gametophyte was higher in the A33 (32.6%) than in the Kh line (23.5%).

INTRODUCTION

Development of the female gametophyte has been the subject of numerous studies. However, there still is an insufficient amount of data concerning embryo sac development and the events leading to seed production. This is especially true for species grown commercially as food plants, e.g. the tomato, a common vegetable.

The development of the embryo sac and its morphology in *Lycopersicon esculentum* are being investigated as part of a study to determine the reason of seed reduction in crosses of this species.

MATERIAL AND METHODS

Two lines of tomato (*Lycopersicon esculentum* L. Mill.): Kholodostoykiye (Kh) and A33 growing in the experimental station at Wolica, AR-SGGW, Warsaw, were used for the study. Line Kh is fully fertile while A33 shows a tendency to parthenocarpy.

Whole ovaries and ovules dissected from ovaries in 2% glutaraldehyde were placed immediately in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1 h and then in fresh 5% glutaraldehyde in the same buffer at room temperature. After overnight fixation the material was dehydrated in alcohols and embedded in Epon 812. Semithin sections cut on an LKB Ultratome after brief staining in 1% toluidine blue were examined under a light microscope and photographed. A total of 1 000 ovaries were examined.

The dimensions of ovules and embryo sacs before anthesis were estimated with a Zeiss ocular planimeter. A total of 200 ovules and embryo sacs were measured.

RESULTS

The female gametophyte in *Lycopersicon esculentum* develops according to the *Polygonum* type.

The ovule is anatropous and unitegmic. Prior to the onset of meiosis the meiocyte, being a single hypodermal cell (Fig. 1) is distinguishable from the other cells of the nucellus only by its size and position (Fig. 2). At this stage of development in some percentage of ovules of the A33 line transparent spaces appear around the meiocyte (Fig. 3). Sometimes large vacuoles can be seen inside the meiocyte cytoplasm (Fig. 4).

The meiocyte by two successive meiotic divisions yields a linear tetrad of megaspores (Figs. 5-8). In the ovules of the A33 line instead of linear arrangement, we observed sporadically meiocytes in the form of a reversed T. Besides lack of cell wells between four haploid nuclei in the meiocyte of line A33 (Fig. 11) no anomalies in meiotic division were seen in both studied tomato lines.

While three megaspores degenerate, the fourth (a chalazal one) increases in size (Fig. 9) and becomes the one-nucleate embryo sac (Fig. 10). In the ovules of lines A33 and Kh we noticed a marked vacuolisation of tetrad cells (Fig. 12).

At the nuclear stage of embryo sac development (Fig. 14) both in the A33 and in the Kh line a distinct degeneration of protoplast occurs in some ovules. In such ovules the whole embryo sac cavity is filled with a mass of protoplast containing numerous vacuoles (Fig. 17). Sometimes the space normally occupied by one embryo sac is divided into two to four compartments separated from one another by thin membranes staining with toluidine blue (Fig. 13). Frequently, they are swollen, wide and irregular in shape, and may contain single nuclei.

Despite the anomalies described above, in the majority of ovules in both studied tomato lines, the cellular embryo sac is formed (Figs. 15-16). At maturity, the embryo sac is long, ellipsoidal, and highly

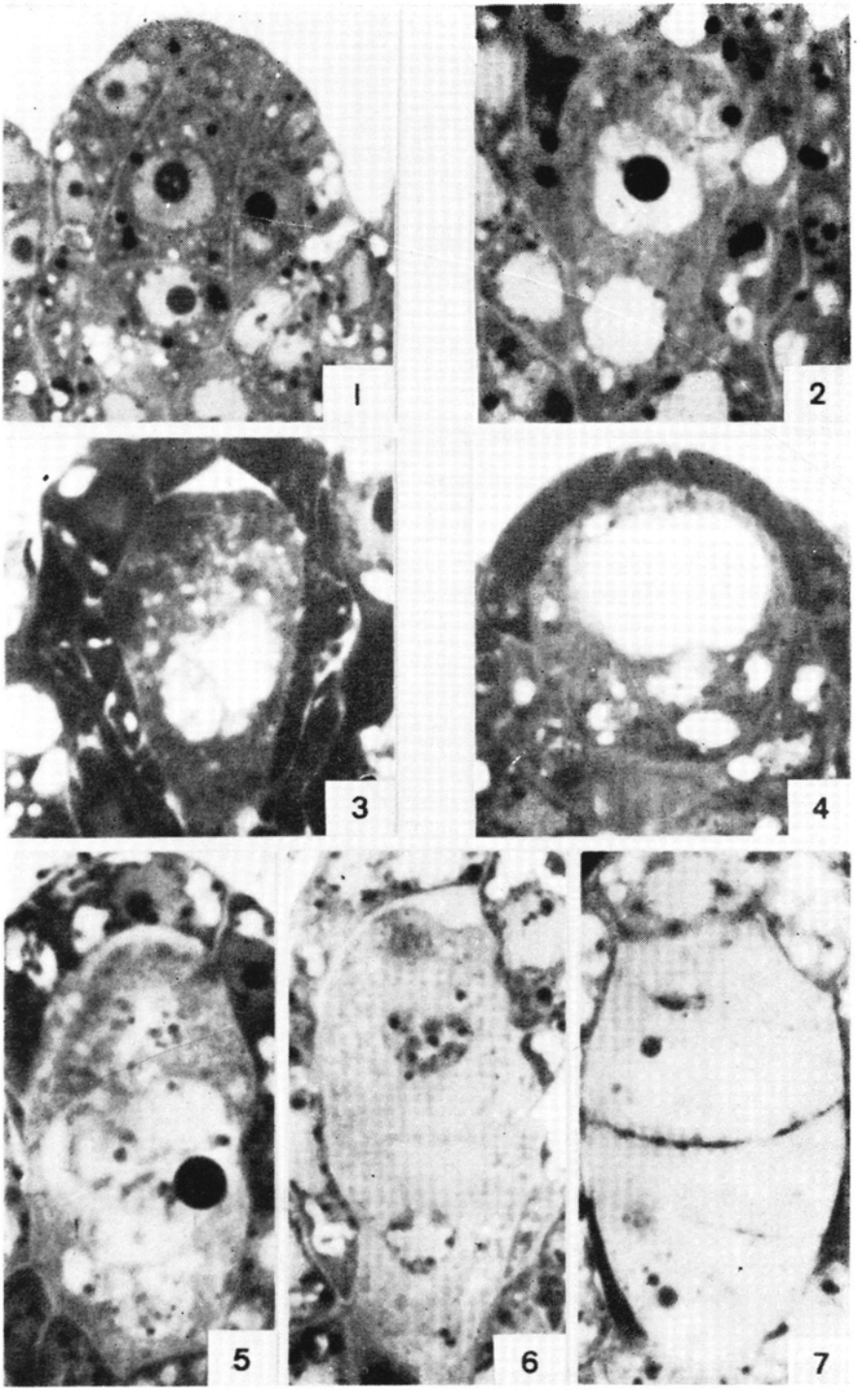

Fig. 1. Kh line. A single hypodermal cell near the tip of the nucleus is distinguishable from the other cells by its size and position. × 2000

Fig. 2. Kh line. Meiocyte beings to enlarge. × 2000

Fig. 3. A33 line. Stage as in Fig. 2. Transparent areas in some places around meiocyte. × 2000

Fig. 4. A33 line. Large vacuole in central part of meiocyte. × 2000

Figs. 5-7. Kh line. Stages of meiosis. × 2000

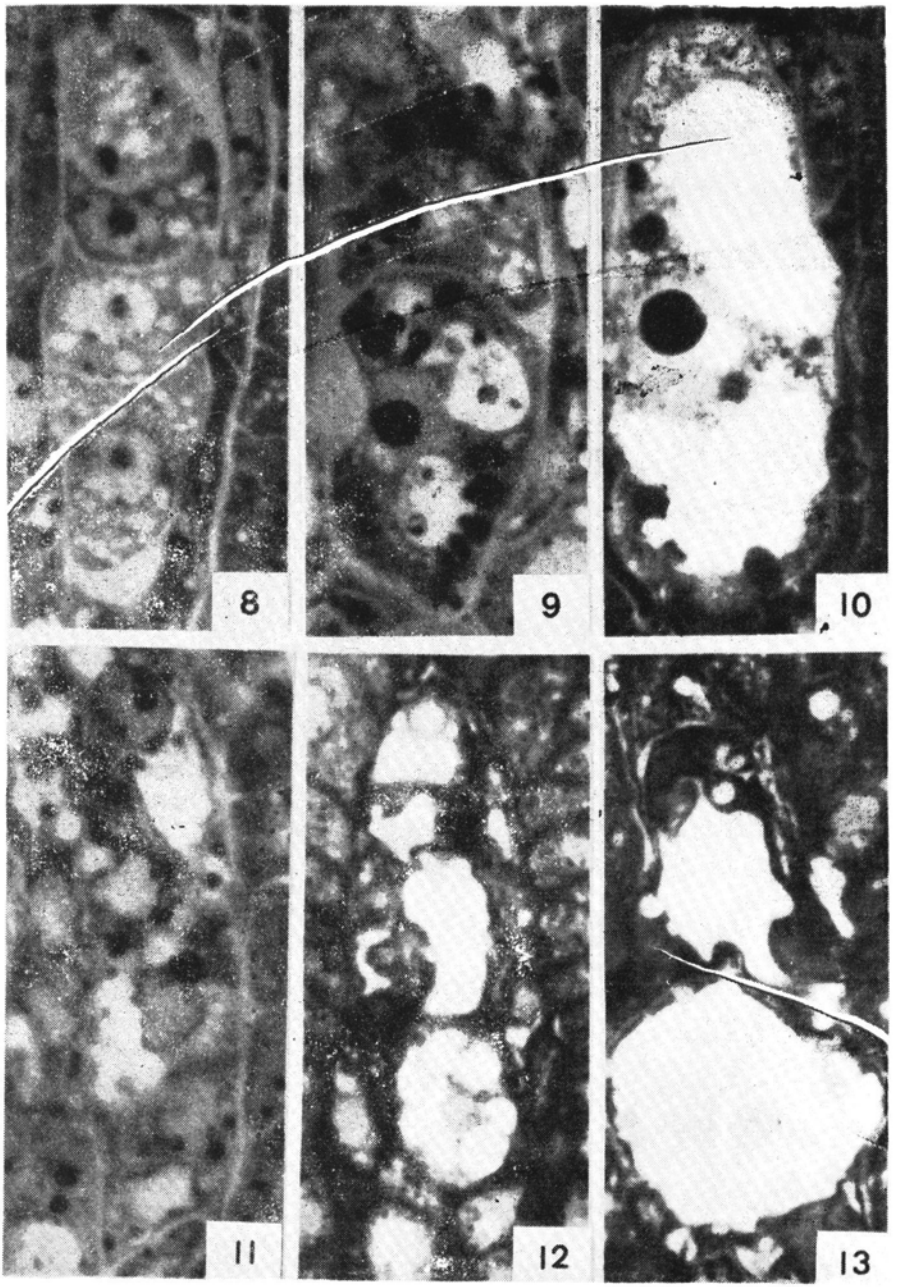

Fig. 8. Kh line. Linear arrangement of tetrad cells. × 2000
Fig. 9. Kh line. The chalazal cell of the tetrad becomes embryo sac mother cell. × 2000
Fig. 10. Kh line. One-nucleate embryo sac. × 2000
Fig. 11. A33 line. No cell walls between post-meiotic nuclei. × 2000
Fig. 12. A33 line. Distinct vacuolisation of tetrad cells. × 1200
Fig. 13. A33 line. Two compartments within space of embryo sac. × 1600

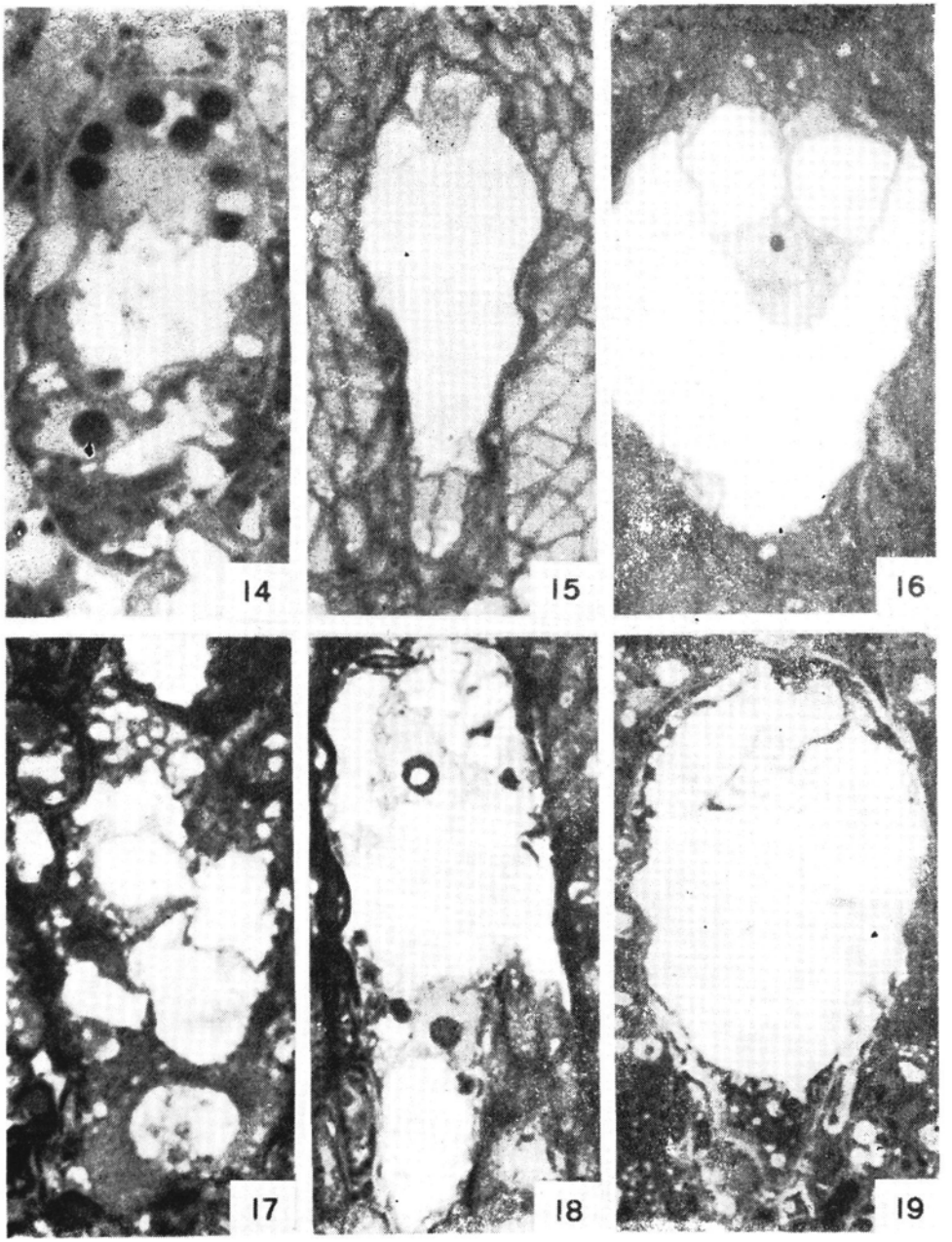

Fig. 14. Kh line. Four-nucleate embryo sac. Large vacuole is present between two groups of nuclei. × 2000

Figs. 15-16. Kh line. Mature embryo sac. Egg apparatus and antipods are visible. Fig. 15 — × 450, Fig. 16 — × 1200

Fig. 17. A33 line. Nuclear stage of embryo sac development. Degeneration of protoplast occurs. × 1600

Fig. 18. A33 line. Degeneration of synergids. Nuclei of egg cell and central cell still visible. × 1350

Fig. 19. A33 line. Remnants of egg apparatus and antipods are present. × 1000

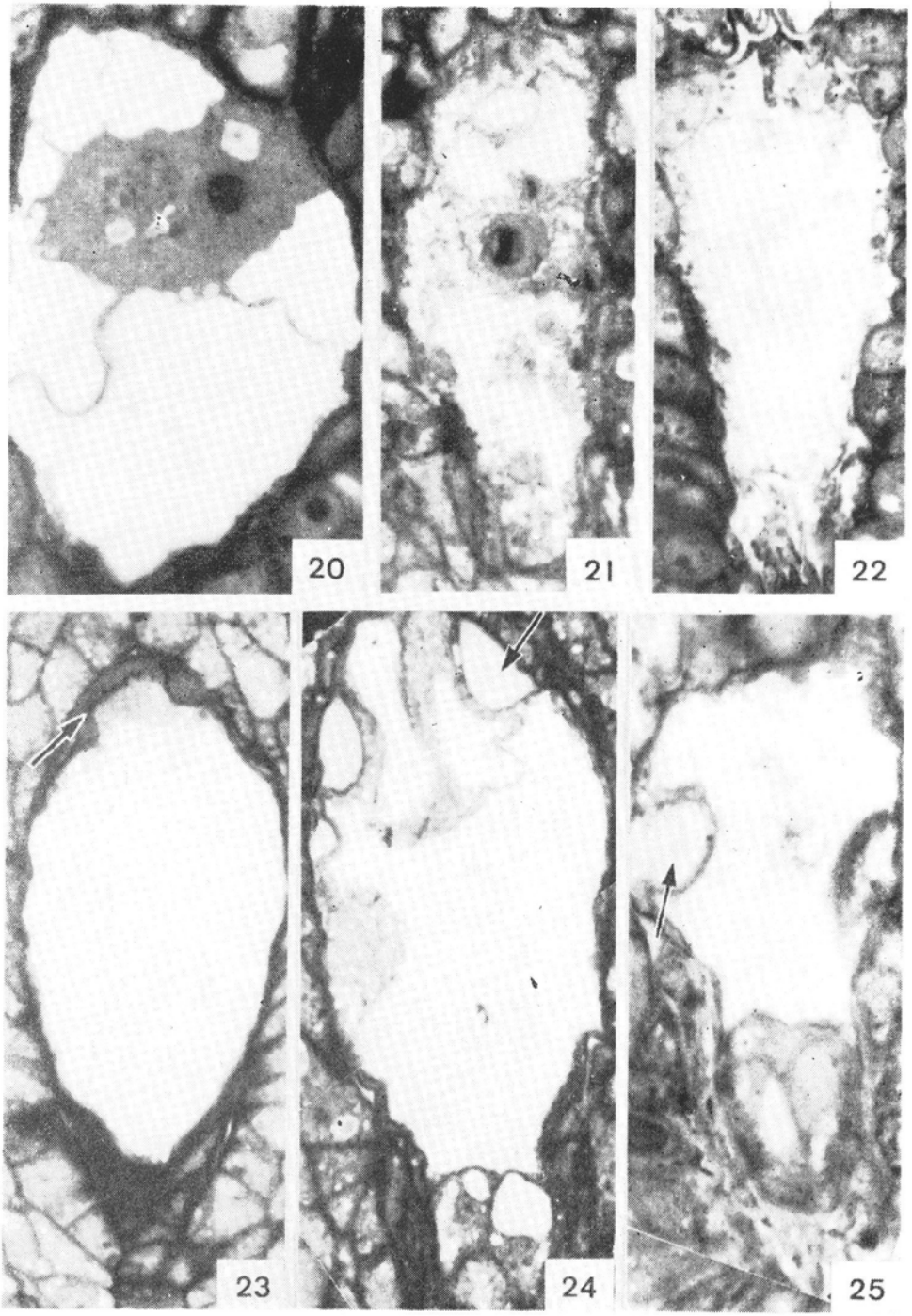

Fig. 20. A33 line. Central cell well visible in almost empty embryo sac cavity. × 1350

Figs. 21-22. A33 line. Later stages of degeneration than in Fig. 20. × 1000

Figs. 23-25. A33 line. Wall projections (arrows) can be seen at the micropylar end and/or in central region of the embryo sac. × 1000

vacuolated. The chalazal tip of the sac is surrounded by a cup-shaped hypostase. The ovules and sacs of A33 and Kh lines are of similar dimension before anthesis (Table 1).

Table 1

Average dimensions of ovules and embryo sacs in two tomato lines before anthesis (in μm)

	Tomato line	
	Kh	A33
Ovules		
Length	203.17±2.71	196.19±3.64
Width	135.01±2.23	136.39±3.69
Embryo sacs		
Length	69.94±2.83	73.07±2.60
Width	26.67±1.64	29.40±1.67

The mature embryo sac has a normal appearance. It contains a well formed egg apparatus, antipods (Figs. 15-16) and central cell.

Signs of progressive disorganization in the embryo sac structure, however, were noticed in ovules of both lines (Figs. 18-25). The synergids and egg cell are the first to undergo degeneration (Figs. 18-19). While the entire egg apparatus has collapsed completely, the central cell (Figs. 18, 20) and three antipodal cells are healthy (Figs. 24—25). Finally, the central cell degenerates (Fig. 21) and the cytoplasm of the mature embryo sac shows an advanced stage of degeneration (Fig. 22).

The process of cell degeneration described above is accompanied by changes in the wall appearance around the embryo sac. At the micropylar end of the gametophyte and/or in its equatorial plane marked wall projections occur in the embryo sac of A33 line (Figs. 23-25).

Table 2

Frequency of disturbances in development of the female gametophyte in two tomato lines (in %)

Stage of development	Tomato line	
	Kh	A33
Pre-meiosis	not observed	2.1
Meiosis	not observed	1.0
Tetrad	1.0	3.0
Nucleate-embryo sac	1.5	3.0
Cellular-embryo sac	21.0	23.5

The frequency of disturbances in the particular stages of embryo sac development described above varied in both tomato lines (Table 2). As indicated in Table 2, essential differences occur in pre-cellular stages

of embryo sac development. In the cellular embryo sac there is no significant difference in the frequency of degenerated sacs in both lines. However, the total number of ovules characterized by aberrant female gametophyte is higher in A33 than in Kh line and reaches 32.6% as compared with 23.5% in Kh line.

DISCUSSION

In the course of the present investigations various disturbances in embryo sac development in two tomato lines were noted. They consisted chiefly in the appearance of transparent spaces around the meiocyte and cell vacuolisation, lack of cell walls between four haploid nuclei, formation of compartments, degeneration of four-nucleate and mature embryo sac. Disturbances in the development of female gametophyte have been also described in other plants e.g. *Acer* (Haskall and Postlethwait 1971), *Carex* (Grężicka 1964), *Lantana* (Tasneem and Nalini 1972), *Quercus* (Mogensen 1975), *Utricularia* (Siddiqui and Farooq 1965).

The disturbances may occur in particular plants at different stages of embryo sac development. For example, as a consequence of rapid disintegration of the micropylar megaspore and of occasional omission of the second meiotic division in the micropylar dyad cell a lack of tetrad was observed in the ovules of *Acer saccharinum* (Haskall and Postlethwait 1971). In avocado ovules the megaspores instead of degenerating continue to develop and form together with the chalazal megaspore compartments and cavities (Tomer and Gottreich 1976). Moreover, degeneration of the four-nucleate embryo sac was noticed in some avocado ovules. Disturbances were also seen in eight-nucleate embryo sacs of *Lantana aculeata* (Tasneem and Nalini 1972).

In genetically unfruitful tomato plant Rick (1946) distinguished two types of ovules: collapsed, with embryo sac cavity occupied by a mass of degenerating gametophyte, and the substitution type, where the sac cavity is filled with a mass of 20 to 40 undifferentiated cells. The pictures presented in this paper (Figs. 13, 17) seem to represent these types of ovules.

All disturbances in the development of the female gametophyte lead usually to ovule abortion (Rick 1946, Walker 1947, Sachar and Kanta 1958, Siddiqui and Farooq 1965, Bhandari 1968, Mogensen 1975). As the consequence of ovule abortion the number of seeds per one fruit is reduced.

The ovaries of two tomato lines: Kholodostoykiye and A33 contain approximately 400 ovules (Gabara and Kubicki, in preparation). Since

the percentage of ovules which develop disturbed tomato embryo sacs is 23.5 (Kh) and 32.6 (A33), one can calculate that about 100 ovules per one ovary will undergo abortion. Nevertheless, about 300 seeds per one fruit should be formed in both tomato lines. Contrary to the above expectation we found an extremely low number of seeds in the A33 line (Gabara and Kubicki, in preparation). Therefore, the irregularities in embryo sac development leading to ovule abortion and described in the present paper cannot be responsible for seed reduction in the line A33.

We do not know the mechanism of ovule abortion in tomato, although it was found that abortive ovules, e.g. in *Anemone*, appear as the consequence of failure of the embryo sacs to complete its development (Bhandari 1968). Ovule abortion in *Malus* seems to be the result of a poorly developed placenta which is unable to support functional ovules (Simons 1974). Rembert (1977) suggests that mechanical repression by the pericarp my be a contributing factor in ovule abortion in *Glycine*. In Rick's (1946) opinion abortion in tomato occurs owing to ovule starvation because of their unfavourable position or of crowding in the ovary.

Most authors consider that the wall projections which develop in a number of species (Marinos 1970, Newcomb and Steeves 1971, Newcomb and Fowke 1973, Schulz and Jensen 1974) increase the area of cell wall across which nutrients can pass into the embryo sac. It cannot be excluded, therefore, that the extremely conspicuous wall projections observed in some percentage of embryo sacs of line A33 would appear as a symptom of female gametophyte defence against starvation. However, no such conspicuous wall projections appeared in line Kh, although degenerated sacs were also numerous in this line.

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Zakłócenia w rozwoju gametofitu żeńskiego u pomidora (Lycopersicon esculentum L. Mill.) całkowicie płodnego i wykazującego tendencję do partenokarpii

Streszczenie

Wykazano, że część zalążków u dwóch linii *Lycopersicon esculentum:* Chołodostojkije (Ch, płodnej) i A33 (z tendencją do partenokarpii) wykazuje zakłócenia w rozwoju woreczka zalążkowego. Te nieprawidłowości mogą być widoczne w czterech fazach: przed-mejotycznej, po-mejotycznej (w tetaradzie), jądrowej i komórkowej. Większość nieprawidłowości obserwowano w stadium komórkowym woreczka zalążkowego. Całkowita liczba zalążków, które wykazują zakłócony rozwój gametofitu żeńskiego, była większa w linii A33 (32,6%) aniżeli w linii Ch (23,5%).