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Factors affecting the production of seeds in fully fertile tomatoes (*Lycopersicon esculentum* L. Mill.) and those showing a tendency to parthenocarpy

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Abstract

Comparative studies on the development of the female gametophyte, pollination and fertilization in two lines of *Lycopersicon esculentum*, Kholodostoykye (Kh, fertile) and A33 (with a tendency to parthenocarpy) have revealed that seed production is affected by disturbances in embryo sac formation but mainly by its degeneration after anthesis, which is especially visible in line A33. Moreover, delayed development of some embryo sacs and incomplete pollination due to various stigma levels seem to be responsible for the diminution of seed number in line A33. Deep fluorescence of numerous pollen grains as well as whole pollen tubes in 83.3 per cent of A33 stigmas and only 24.1 per cent in the Kh line points to the heterogeneity of pollen. This could be one more reason for reduced fertility. The results of application of plant growth regulators (auxin, PCIB) which affect seed production in tomato of line A33 remain inconclusive.

Key words: seed production, tomato, growth regulators

INTRODUCTION

In Poland, the production of early tomatoes under field conditions, in spite of the low temperature of early season, appears to be a very important problem. The tomato of A33 line obtained in the Department of Genetics and Plant Breeding, AR-SGGW in Warsaw, by selection of F_2 plants of *L. esculentum* var. Earlinorth x *L. esculentum* var. Beaverlodge

seems to represent such tomatoes. However, seed production in this new line of tomato is very low in comparison with fertile plants.

Among the processes that are responsible for seed formation, two appear the most essential: 1) the development of the female and male gametophytes, 2) fertilization. Anomalies in one or in both these processes usually lead to low seed production or even to formation of seedless fruits. i.e. to plant sterility.

In an earlier paper of this series we have described the formation of the embryo sac and the disturbances in its development in two lines of tomato: Kh, fertile and A33, showing a tendency to parthenocarpy (Gabara and Kubicki 1983). The present paper deals with the processes associated with pollination and fertilization in the studied tomato lines.

MATERIAL AND METHODS

Two lines of tomato (*Lycopersicon esculentum* L. Mill.), Kholodostoykye (Kh, fertile) and A33 (with a tendency to parthenocarpy) grown at the experimental station at Wolica, AR-SGGW, Warsaw, were used for this study.

Flowers used for studying the structure of the female gametophyte were fixed as previously described (Gabara and Kubicki 1983).

Male fertility (viable pollen) was estimated as the percentage of pollen stainable with acetocarmine. The number of pollen grains which had germinated in 2% agar containing 15 per cent sucrose and 0.15 g per 1 boric acid was measured after 4 h cultivation (Mc Leod 1975).

To check whether the partial sterility in A33 line is caused by a failure in pollinantion and/or fertilization, we followed the germination of pollen, growth of pollen tubes in the stylar and ovarian tissues using the modification of the staining method of Eschrich and Currier (1964) and fluorescence microscopy. Pollen tube growth was studied by taking flowers at anthesis and 2-6 days after anthesis and fixing them in FAA (formalin, acetic acid, ethanol, 1:1:18) for 24 hours. After rinsing with water and separating the perianth from the ovaries, pistils were macerated in 5 N NaOH for 24 h and stained for 6-12 h with 2% aniline blue in 20% K_3PO_4 solution (Wilms 1974). The stained pistils were squashed gently in glycerol and pollen tube growth was estimated by UV fluorescence microscopy.

Plant growth regulators were applied as aqueous solutions prepared freshly each time. The compounds used were: Naphtoxyacetic acid, Gibrescol (GA_3) and PCIB, at the concentrations of 1000 ppm: they were sprayed on bud flowers of line A33. In addition, hand pollination of emasculated flowers of this line was done.

RESULTS AND DISCUSSION

The ovary in line A33 contains about 17.8 ovules less than that in the fertile line (Table 1). As is indicated from our previous observations (Gabara and Kubicki 1983), only 32.6 per cent of ovules in line A33 showed disturbances in the development of the female gametophyte. Therefore, the high number of ovules per ovary in line A33 supports our earlier assumption (Gabara and Kubicki 1983) that disturbances in the female gametophyte development cannot be responsible for low seed production in this line. A low percentage of irregularities in the development of the female gametophyte cannot determine the number of seeds even in fertile tomatoes, since the ovule number per ovary in this line was 446.3 ± 23.4 and the number of seeds only 195.4 ± 18.9 (Table 4). Taking into consideration the above data it was reasonable to suppose that disturbances in pollination and/or in fertilization cause the reduction of seeds in line A33 and affect formation of one half of the seeds in fertile plants as calculated on the basis of ovule number per ovary.

After anthesis the number of ovules showing abnormal, degenerating female gametophyte increased to 32.9% in the seeded line and to 71.7% in line A33 (Table 1).

Table 1

Characteristic of ovules in two tomato lines: Kh (fertile) and A33 (with a tendency to parthenocarpy)

Line	Number of ovules per ovary	Percentage of ovules with irregularities in the gametophyte development	
		in flower buds*	in fully expanded flowers (6 days after anthesis)
Kh	446.3 ± 23.4	23.5	32.9
A33	379.0 ± 16.1	32.6	71.7

* Gabara and Kubicki (1983).

At the time of anthesis, three types of flowers can be distinquished in both tomato lines: the first, with the stigma below the mouth of the anther tube, the second, where the stigma is on the same level as the mouth of the anther tube, and the third, where the orifice of the anther tube is situated below the stigma (Table 2). In the fertile line, the first type of flower dominates, i.e. with the stigma depressed below the mouth of anther tube (89.8%). The lower stigma level results from a reduced rate of elongation of the style (Rick and Dempsey 1969). In line A33 only 48.2% of flowers at anthesis have stigma below the orifice of the anther tube. It is interesting that in many flowers of this line. although the length of anthers and style appears similar, the stigma is well visible

due to separation of the tips of united anthers. Such flowers were classified as belonging to the third group of flowers.

Anthers in tomato dehisce a few hours before anthesis, therefore pollination occurs when the stigma is present inside the anther tube. This was well visible in the fertile tomato line (Kh), where 72.1% of flowers with stigma situated below the orifice of the anther tube had already been pollinated (Table 2). In line A33, however, pollination took place a little later, i.e. when the stigma reached the level of the mouth of the anther tube, even in flowers with stigma visible among the separated tips of anthers (Table 2).

Table 2

Position of the stigma and number of pollinated flowers (in brackets) at anthesis in two tomato lines: fertile (Kh) and showing a tendency to parthenocarpy (A33). Each reading represents an average of measurements from 200 flowers

Stigma position	Kh	A33
Below tube of anthers	89.8 (72.1)	48.2 (19.2)
At the level of orifice of the anther tube	5.7 (26.5)	14.1 (49.0)
Above tube of anthers	4.5 (1.4)	37.7*(32.8)

* The stigma is well visible due to separated tips of the anther tube

It should be stressed, that pollination in this group of A33 flowers appears incomplete, since numerous pistils have only a small amout of pollen grains at the stigmatic surface. Rick and Dempsey (1969) have also noticed heavier and more consistent pollen coverage of the styles with low stigms. In these authors' opinion, the low stigma level ensures pollination because the stigma not only is more accessible for pollination, but also does not tend to block the flow of pollen. The stigma may plug the orifice of anther tube so effectively that even hand pollination fails to effect pollen flow (Rick and Dempsey 1969). It seems reasonable to suppose that incomplete pollination due to stigma level in relation to the mouth of anther tube may be responsible for the very high percentage of degenerated female gametophytes in A33 after anthesis and in consequence of that, for the low seed production (Table 4).

No disturbances were visible in microsporogenesis in both tomato lines or in the dehiscence of anthers.

The pollen germination test on artificial medium showed up to 65.8 per cent germination of fresh pollen in line A33 which was even higher than in the fertile line, where germination of pollen reached a value of 47.8 per cent. Pollen viability estimated by acetocarmine staining was similar in both tomato lines (Table 3).

Differences exist between the two studied tomato lines in the cause of progamic phase (Figs. 1-12). Although germination of pollen at the stigmatic surface seemed to be normal (Figs. 1, 7), besides pollen tubes characterized by small, widely spaced callose plugs, numerous germinating pollen grains and whole pollen tubes showed deep fluorescence with aniline blue (Fig. 7). Approximately 83.3 per cent of stigmas in line A33 revealed the presence of such pollen and only 24.1 per cent of stigmas in the fertile line.

Table 3

Percentage of viable and germinated pollen grains in two tomato lines: fertile (Kh) and showing a tendency to parthenocarpy (A33)

Line	Viability	Germination
Kh	92.7	47.8
A33	94.6	65.8

Table 4

Effect of hormones and hand pollination on the number of seeds per fruit in two tomato lines: Kh (fertile) and A33 (showing a tendency to parthenocarpy). Each reading represents an average of measurements from 20 plants

Material	Kh	A33
Not treated	195.4±18.9	32.6±3.8
Naphtoxyacetic acid		68.9±3.8
Gibrescol (GA_3)		34.2±4.0
PCIB		70.7±6.9
Hand pollination		46.0±3.8

In both lines, the numerous pollen tube grow rapidly down the style (Figs. 2, 8), then enter into the ovary, where they surround particular ovules (Fig. 3). Although it is obvious that natural variation in the number of pollen grains deposited on stigmas lead to variance in seed number among the fruits on a given individual, generally, in line A33 pollen tubes in the style and ovary are less numerous than in the fertile line.

In line A33 we also noticed abnormal retardation of tube growth. Pollen tubes coiled rather than entering the embryo sac in a way similar to that described in the interspecific crosses of *Medicago* (Sangduen et al. 1983). The family *Solanaceae* is characteristic of a gametophytic self-incompatibility system, where the rejection reaction of the incompatible pollen tube occurs in the stylar transmitting tissue (Shivanna et al. 1982). It is difficult to believe, that a part of the pollen in the line A33 and some in line Kh represents an incompatibility system.

In many ovules of fertile tomato plants, an ingrowth of pollen tubes into the nucellus was observed (Figs. 4, 5). Moreover, in a few cases, the branching of tubes could be noticed in the ovary of the fertile line (Fig. 6). Branching of pollen tubes observed in spinach (Ramanna and Mutsaerts 1971, Wilms 1974), avocado (Sedgley 1979) and in the interspecific crosses of *Medicago* (Sangduen et al. 1983) has been atributed to abnormal pollen tube — style reaction. On the other hand, in Wilms' (1974) opinion, tube branching can be seen as a postfertilization process.

Pictures of tube ingrowth into the nucellus were very rare in line A33. Most of the ovules at this time demonstrated a strong fluorescence of the embryo sac (Figs. 10, 11). The contents of such embryo sacs as seen in the light microscope show advanced stages of degeneration. In a few percent of the ovules, we observed only the tetrad stage. (Fig. 9) which suggests delayed development of the embryo sac in line A33. Fluorescence of nucellar cells could also be noticed in some ovules of line A33 (Fig. 12).

Hand pollination very slightly increased seed number in fruits of line A33 (Table 4). Application of gibberelin had no effect on this process. It is noteworthy, that antiauxin — PCIB — caused an increase of seed number to about 116 per cent. This latter observation permits the suggestion that a too high auxin level in the pistil of line A33 could be one of the reasons for partial female sterility in the tomato, which is in agreement with Gustafson's (1939) data. This author has stated that ovules of fruit cultivars able to develop parthenocarpically were richer in auxin activity (at anthesis) than cultivars which require fertilization and seed development for fruit growth. In light of the above assumption, however, it is not clear why auxin alone also increase the number of seeds in the fruits of A33 line.

We hope that further experiments with hormones will supply new data for better determining the factors affecting low seed production in the tomato of line A33.

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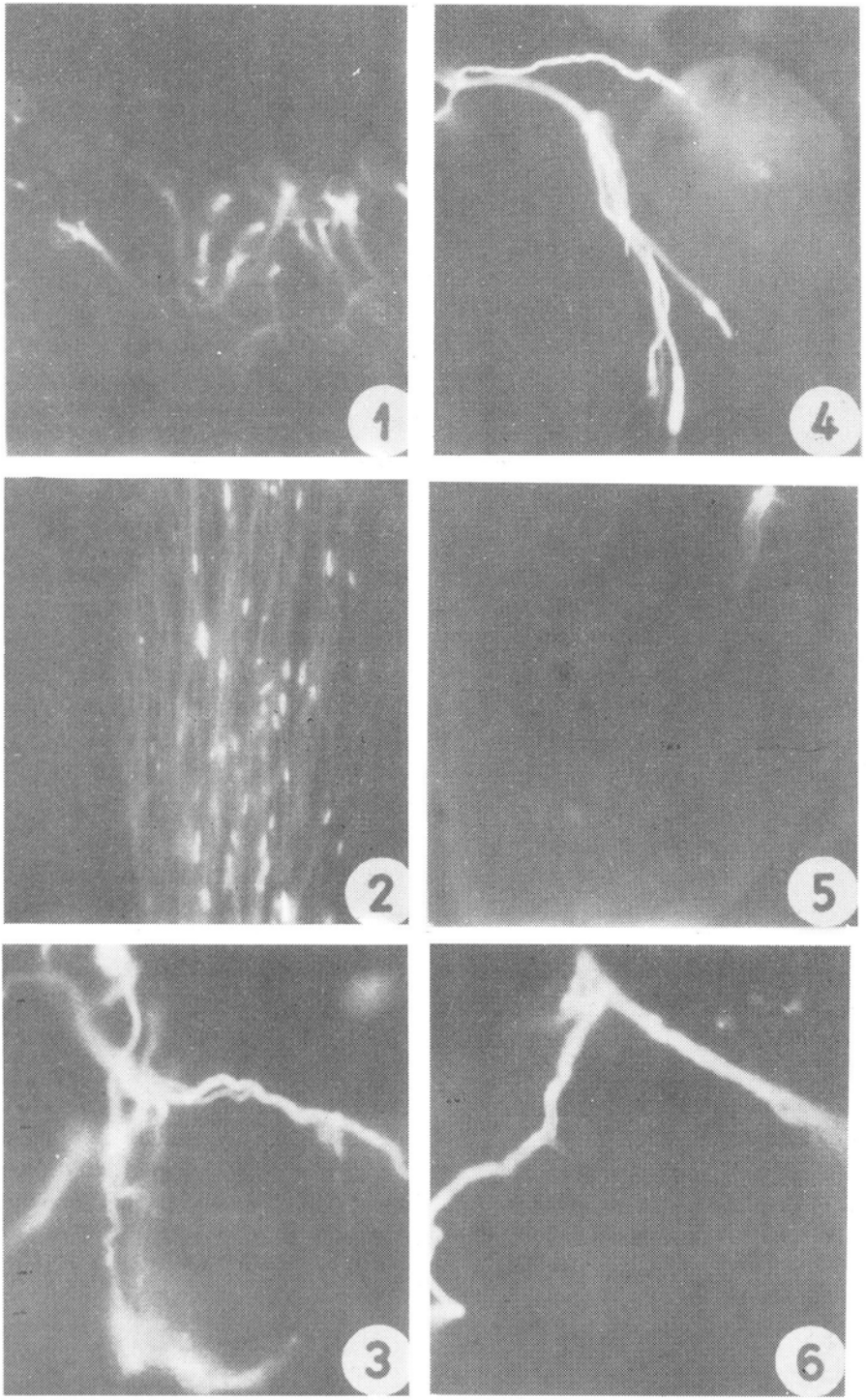
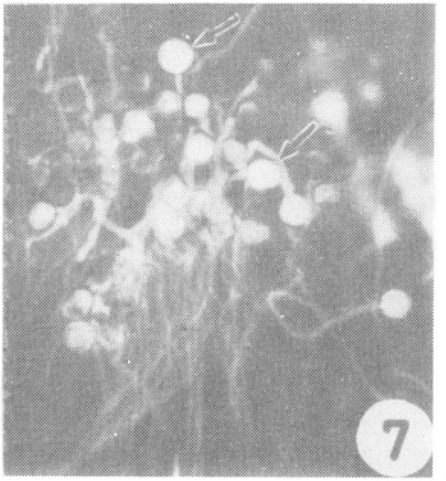
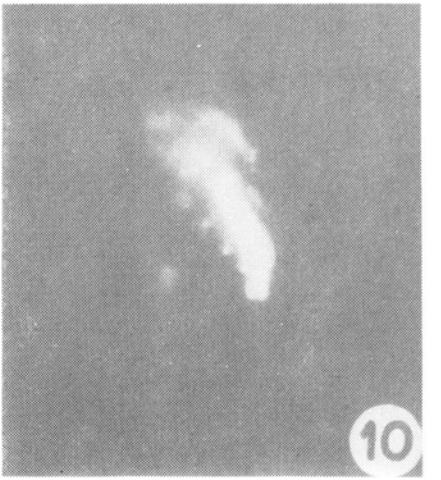
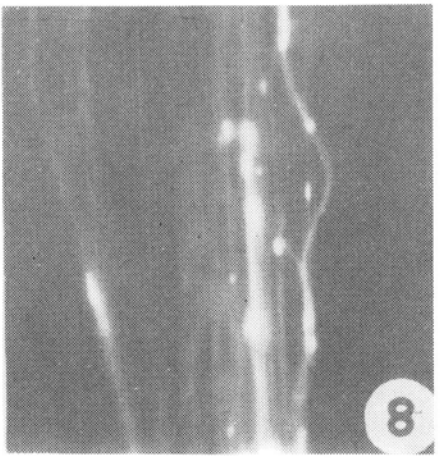
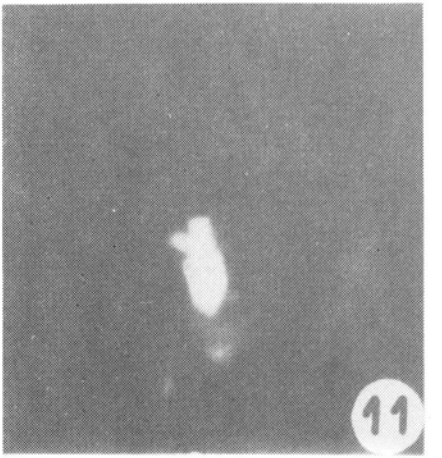


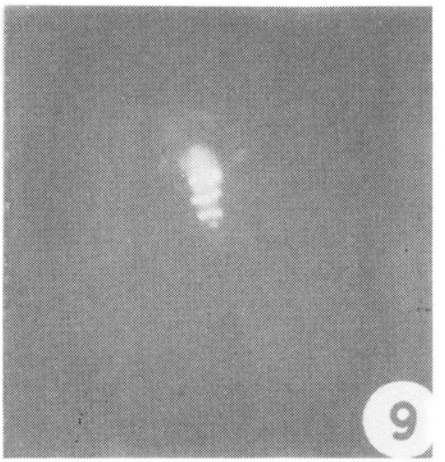
Figs. 1-6. Kh line. Fig. 1. Pollen grains germinating at the surface of stigma. X 350. Fig. 2. Numerous pollen tubes characterized by small widely spaced callose plugs are visible inside the style of fertile tomato. X 350. Figs. 3-6. Pollen tubes in ovary of fertile tomato, surrounding particular ovules (Fig. 3), entering into ovules (Figs. 4, 5). Short branched pollen tubes are also visible (Fig. 6). Figs. 3, 4 — X 350, Figs. 5, 6 — X 500

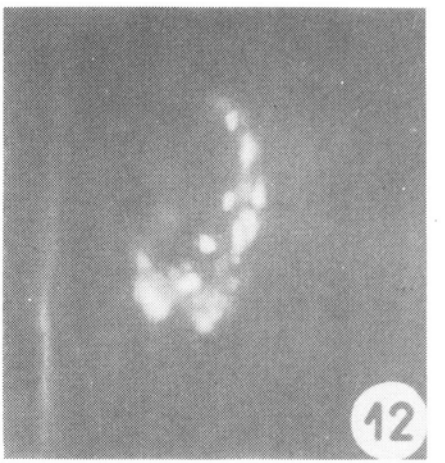












Figs. 7-12. A33 line. Fig. 7. Two types of germinating pollen grains are present at stigmatic surface in the majority of flowers: the first, showing the presence of callose plugs, and the second, whole pollen grains and pollen tubes exhibit a strong fluorescence after aniline blue treatment (arrows). X 350. Fig. 8. Pollen tubes inside the style. They are less numerous than in fertile line. X 350. Fig. 9. Tetrad stage in an ovule with delayed development of embryo sac. X 450. Figs. 10-11. Degenerated embryo sacs showing strong fluorescence. X 450. Fig. 12. Fluorescence of nucellar cells is well visible in some ovules. X 500

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Czynniki wpływające na produkcję nasion u pomidorów (Lycopersicon esculentum L. Mill.) całkowicie płodnych i wykazujących tendencję do partenokarpii

Streszczenie

Badania porównawcze rozwoju gametofitu żeńskiego, zapylenia i zapłodnienia u dwu linii *Lycopersicon esculentum*: Chołodostojkije (Ch, płodnej) i A33 (z tendencją do partenokarpii) wykazały, że na produkcję nasion wpływają zakłócenia w rozwoju woreczka zalążkowego a głównie jego degeneracja po otwarciu kwiatu, jest to szczególnie widoczne u linii A33. Ponadto, opóźniony rozwój niektórych woreczków zalążkowych i niecałkowite zapylenie spowodowane różnym położeniem znamienia wydają się być odpowiedzialne za spadek liczby nasion u linii A33. Silna fluorescencja licznych ziaren pyłku i całych łagiewek pyłkowych w przypadku 83,3% znamion u A33 i tylko 24,1% u linii Ch wskazuje na heterogenność pyłku. To mogłoby być jedną więcej przyczyną obniżonej płodności. Wyniki otrzymane po zastosowaniu roślinnych regulatorów wzrostu (auksyna, PCIB) wpływających na produkcję nasion u pomidora linii A33 są niejasne.