Histological studies on the degenerative process of pollens in sterile peach

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Summary : A study was undertaken to determine the critical phase of the degeneration of pollen grains and tapetum in Hakuto peach.

1) The development of pollen mother cells in Hakuto peach appeared to be quite normal until the premeiotic stage.

2) The degeneration of microspores took place at the vacuole stage, and thereafter, they showed little or no increase in size and resulted in empty medium-sized pollen grains.

3) At the premeiotic stage, the tapetal cells contained a number of amyloplasts and then, they remained intact until the microspores were formed, thereafter, they might lose contact with each other, became highly vacuolate and later degenerated during the course of pollen development.

4) It is concluded that the degeneration of pollen grains takes place at the vacuole stage and is due to abnormal tapetal cells.

Introduction

Cytological and histological studies in the pollen sterility of peach have been carried out by some workers. Asami (1) reported that the majority of pollen grains in Shanghai peach degenerated due to the irregular microspore division and the incomplete development of cytoplasm.

A more detailed study was given in the recently published work by Ozono (4), who investigated the detail of the development of pollen grains and tapetum in Hakuto peach. He pointed out that the degeneration of pollen grains might take place at the vacuole enlarging stage and that the time of occurrence was probably related to time of degeneration of tapetum.

However, the occurrence of male-sterility in Hakuto peach seems to be a very complicated phemomenon.

The present study was undertaken to determine the critical phase of the degeneration of pollen grains and tapetum in Hakuto peach.

Materials and Methods

Materials for this study were about 20 years old Hakuto and Okubo peach trees which are sterile and fertile varieties, respectively.

In order to determine the critical phase of degeneration of pollen grain and tapetum, flower-buds in two varieties were collected from late November, 1972 to late March, 1973.

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For the observation of pollen development under light microscope, flower-buds were fixed in formalin-acetic acid-60% ethanol (5 : 5 : 90v/v). The dehydration, infiltration and embedding in paraffin were carried out according to usual methods. Sections were cut in 10 to 15μ thickness and stained with fuchsine acid and fast green.

For a detailed observation of fine structure in pollen development, the electron microscope was also used. Anthers in two varieties were collected from late Novemder, 1972 to late March, 1973. They were fixed in 4% glutaraldehyde in phosphate buffer, pH 7.2 at 4 °C for 2 hours. After rinsing for 30 minutes with Millonig's phosphate buffer solution, they were post fixed in 1 % OsO_4 solution, buffered at pH 7.2 for 60 minutes. The anthers were dehydrated by passing them through increasingly concentrated aqueous ethanol. They were finally embedded in Epon 812. Sections were cut with glass knives and were stained with uranyl acetate.

Observation and Discussion

Pollen abortion within cultivated varieties of the peach has been described by Knowlton (3), but so far as known, there are few publications about pollen abortion in the peach.

An anther of peach is tetrasporangiate. The young pollen mother cells are surrounded by a simple layer of tapetum, two middle layers, two or three endothecial layers, and a rather thick-walled epidermis. The tapetum layer arises from the peripheral layers of the sporogenous tissue and has uninucleate cell. The middle layer is composed of slightly compressed cells, while cells of endothecium layer are large.

Plate I shows the development and degeneration of tapetum and pollen grains in sterile Hakuto as compared with fertile Okubo peach at the level of the light microscopy.

The stage of the pollen-tetrad in sterile Hakuto was observed earlier by one week as compared with the fertile Okubo peach. The development of pollen mother cells and tapetum in Hakuto appeared to be quite normal until the premeiotic stage (I–1).

Therefore, it is assumed that the degeneration of pollen grains in sterile Hakuto peach does not take place during the development of pollen mother cells.

At the pollen-tetrad stage, abnormal sporads were observed partially (I–2). The degree at which the irregular meiotic divisions in pollen mother cells took place was very low(I–2).

Therefore, the primary cause of pollen abortion in Hakuto peach was not the abnormality in the meiotic divisions of pollen mother cells.

Soon after the microspores were formed at the vacuole stage, degeneration set in. First sign of the degeneration could be recognized in the deeper staining of the cytoplasm, as shown in plate(I–3).

Thereafter, the microspores showed little or no increase in size and resulted in empty medium-sized pollen grains (I–4).

The tapetum in peach belongs to the secrectory tapetum type. The tapetal cells at the stage of pollen mother cells continued to increase in size.

At the stage of pollen-tetrads, the tapetal cells increased in diameter. Soon after the liberation of the microspores from the wall of pollen-tetrads, the tapetal cells of fertile anthers began to degenerate and at the later stage, they decreased in size and became compact and structureless (I-7).

However, the tapetal cells of sterile anthers remained same in appearance after breakdown in the microspores (I-3). Their tapetal cells are not likely to be available for microspores or they interrupt the supply of nutrition.

Plate II shows the development and degeneration of tapetum and pollen grains in sterile Hakuto peach as compared with fertile Okubo peach at the level of electron microscopy. Prior to tetrad formation, little if any morphological difference exists between sterile and fertile anthers observed in the level of electoron microscopy. The behaviors in pollen mother cells of sterile Hakuto appeared normal (II-1.2). The pollen mother cell wall has become much thicker and more electron-dense. At the premeiotic stage, the tapetal cells in fertile Okubo expanded and large vacuoles appeared in the cytoplasm (II-7).

On the contrary, the tapetal cells in sterile Hakuto contained a number of amylopl-asts in the cytoplasm and were abnormal in appearance(II-3). At the pollen-tetrad stage, abnormal sporads were observed partially. Thereafter, the degeneration of microspore took place at the vacuole stage. It is considered that the microspores fail to absorb the precursors or nutrients from the tapetum.

So that, it seems that the primary cause of pollen abortion in Hakuto peach would be attributed to abnormal carbohydrate metabolism of the tapetum at the premeiotic stage.

Recently, Hirata et al. (2) pointed out that the abortion of pollen in Hakuto peach might be closely related to the activities of various enzymes such as amylase, invertase and maltase in the anther at various stages.

It is assumed that the nutrient for pollen growth are derived from the tapetum. Therefore, it may be considered that the degeneration of microspores in Hakuto peach takes place at the vacuole stage and is due to abnormal tapetal cells.

要旨：本実験は，モモ白桃の花粉退化期およびタペート崩壊の時期を明らかにするために行なった。

1. 白桃の花粉母細胞の発達は，全く正常であった。
2. 液胞期に達すると，白桃の小胞子の異常が認められ，その後小胞子の大きさは，ほとんど増加せず，内容物が空虚になって退化した。
3. 花粉4分子直前になると，タペート細胞は，細胞質内に多数のアミロプラストを含み，小胞子が形成された後も，タペート細胞は結合状態のままで，その後大久保に比較して異常であると思われた。
4. モモ白桃花粉の退化は液胞期に起こり，その原因は4分子直前のタペート細胞の異常に由来するものと思われた。

References

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2) Hirata, N., H. Kurooka, and S. Nakagawa. (1966) : Abst. Meeting in Jap. Soc. Hort. Sci. November in 1966.

3) Knowlton, H. E. (1925) : Proc. Amer. Soc. Hort. Sci., 21 : 67-69.

4) Ozono, T. (1969) : The paper as dissertation for degree of M. Agr. at Univ. Osaka. Pref.

Explanation of plate

Plate I shows the development and degeneration of tapetum and pollen grains in male sterile peach as compared with fertile peach at the level of the light microscopy.

Photographs No. 1–4. Development and degeneration of tapetum and pollen grains in Hakuto peach.

1. A cross-section of an anther at the pollen mother cells stage showing pollen mother-cells, tapetum, middle layers, endothecial layers, and epidermis. ×300.
2. Abnormal sporad formation during the pollen-tetrads stage. ×600.
3. The microspores beginning to degenerate at the vacuole stage and the tapetal cells remaining complete. ×300.
4. The behavior in the tapetum after the pollen mitosis stage. ×300.

Photographs No. 5–8. Development of tapetum and pollen grains in Okubo peach.

5. Prior to tetrad formation, the pollen mother cells. ×300.
6. Normal sporad formation during the pollen-tetrads stage. ×600.
7. The microspore formation at the pollen-tetrads stage. ×300.
8. The pollen grains after maturation division. ×300.

E : endothecium. EP : epidermis. M : middle layer. MS : microspore.
PT : pollen tetrad. PMC : pollen mother cell. PG : pollen grain.
T : tapetum.

Plate II shows the development and degeneration of tapetum and pollen grains in male sterile peach as compared with fertile peach at the level of the electron microscopy.

Photographs No. 1–4. Development and degeneration of tapetum and pollen grains in Hakuto peach.

1. The behavior in pollen mother cell in initial development. ×5000
2. The behavior in pollen mother cell at the premeiotic stage. ×5000
3. Abnormal tapetal cell at the premeiotic stage. ×5000.
4. Empty medium-sized microspore at vacuole stage. ×3000.

Potographs No. 5–8. Development of tapetum and pollen grains in Okubo peach.

5. The behavior in pollen mother cell in initial development. ×5000.
6. The behavior in pollen mother cell at the premeiotic stage. ×5000.
7. Normal tapetal cell at the premeiotic stage. ×5000.
8. The pollen grain after the vacuole stage. ×3000.

A : amyloplast. C : chromatin. MT : mitochondrion. N : nucleus.
NU : nucleolus. NUP : nuclear pore. L : lipid droplet. V : vacuole.

Plate I

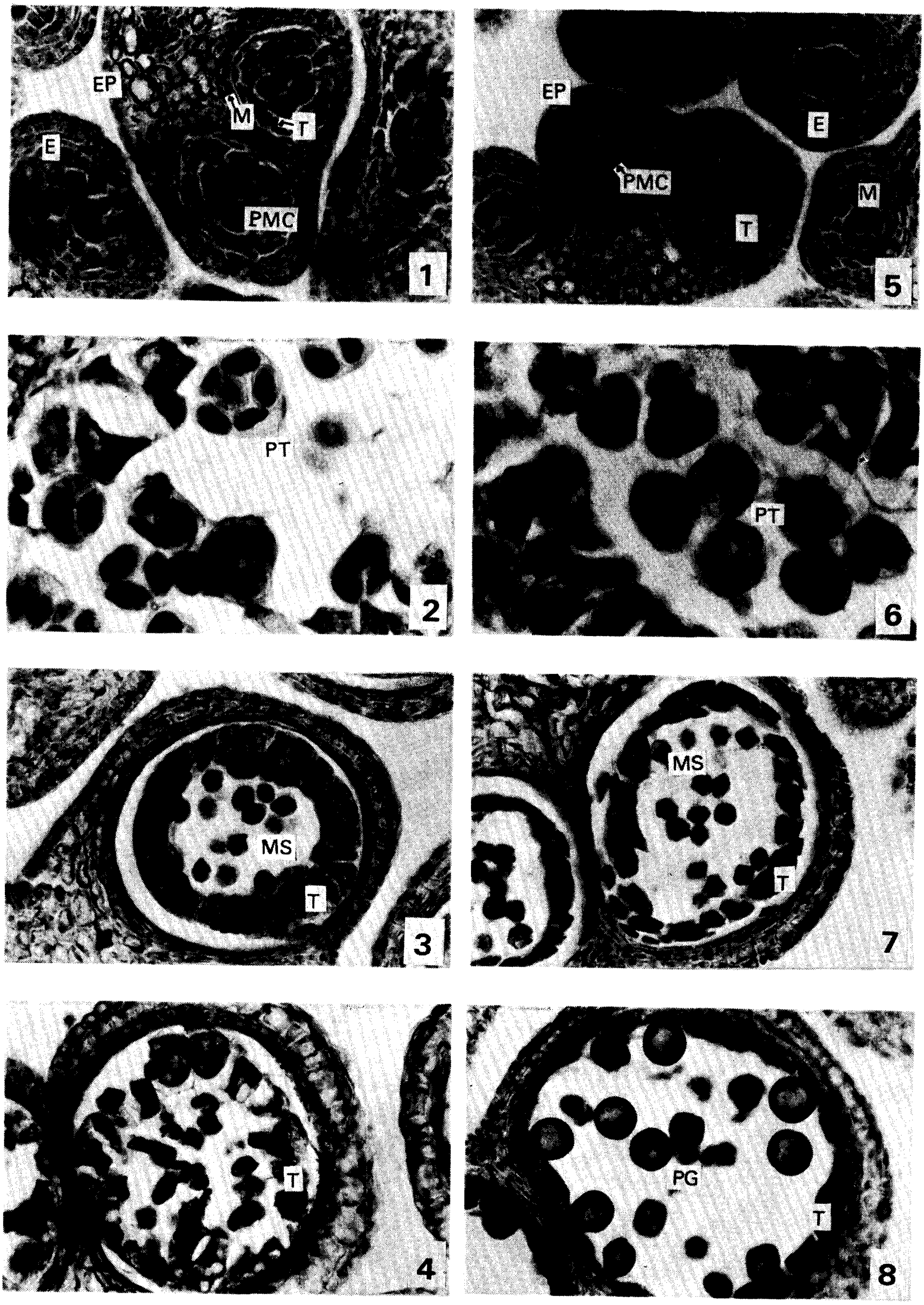

Plate II