Male Sterility Caused by Cooling Treatment at the Young Microspore Stage in Rice Plants

XII. Classification of tapetal hypertrophy on the basis of ultrastructure*

Iwao NISHIYAMA

(Hokkaido National Agricultural Experiment Station, Hitsujigaoka, Sapporo 061-01)

In a previous paper¹⁾, the author reported electron microscopical observations on tapetal cells dilated by cooling treatment at the young microspore stage. At that time the induction of other types of tapetal hypertrophy than hill type was difficult, because the stage of cooling treatments was not just at the young microspore stage but a little earlier. This paper describes an outline of ultrastructures of tapetal hypertrophy for all major types, and presents a new classification of the hypertrophy on the basis of these observations. The details of ultrastructure of hypertrophy for major types will be described in the succeeding paper.

MATERIALS AND METHODS

A paddy rice variety, Hayayuki, was cultured and cooled in natural light rooms of the phytotron. The cooling treatments were made at 12°C for 4 days around the young microspore stage. Anthers were excised from young spikelets during and after the treatments. Both ends of the excised anthers were cut off in 2.5 percent glutaraldehyde solution (buffered with 0.1M phosphate at pH 7.0). The cut materials were fixed at room temperature for 60 minutes in the glutaraldehyde solution under low air pressure made by a tap water aspirator. After washing with 0.1 M phosphate buffer (pH 7.0), they were post-fixed in 1 percent osmium tetroxide (buffered with 0.05 M phosphate at pH 7.0) at 4°C for 60 minutes, washed with water, dehydrated through an alcohol series and propylene oxide, and embedded in Epon resin. Ultrathin sections were stained with 5 percent uranyl acetate for 60 minutes and with Reynolds lead solution²⁾

for 3 minutes.

RESULTS

For hill type hypertrophy of tapetal cells, two kinds were distinguished according to the existence of the primary wall at the inner face of the tapetum. Figs. 1 to 5 illustrate the one kind, namely, hill type hypertrophy "with" primary wall. Figs. 6 to 8 illustrate the other kind, namely, hill type hypertrophy "without" primary wall.

Fig. 1 shows the initial phase of hill type hypertrophy with primary wall. Two tapetal cells in the center dilated. One normal and four broken tetrad microspores are also seen in the locular cavity, with the remnant callose substance. Fig. 2 shows a typical hill type cell with primary wall. This cell consists of four original tapetal cells (in the face of this photograph). The amount of cytoplasm increased with dilatation. Broken microspores are seen in the locular cavity.

Fig. 3 shows a part of a large hill type cell with primary wall. A part of a microspore is on the top right. A part of a degenerating hill type cell with primary wall is shown in fig. 4. The cytoplasm of the dilated cell partly condensed and partly diluted. The accumulation of lipids into granules shows the proceeding of autolysis. In the locular cavity, floating tapetal cytoplasm is seen. This cytoplasm is considered to have originated from balloon type hypertrophy.

Fig. 5 shows a part of a large hill type cell with very thin primary wall at the inner face of the tapetum.

Figs. 6 and 7 show typical hill type hypertrophy without primary wall. The dilated cell in fig. 6

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Fig. 1 Initial phase of hill type tapetal hypertrophy with primary wall. A normal (MS) and four broken (BMS) tetrad microspores are seen. T: tapetal cell; DT: dilated tapetal cell. $\times 2,000$

Fig. 2 Hill type tapetal hypertrophy with primary wall. Arrows indicate the broken cell walls (and membranes) which separated original four cells. T: tapetal cell; DT: dilated tapetal cell; BMS: broken tetrad microspore. $\times 2,000$

Fig. 3 A part of a big hill type cell with primary wall. A nascent microspore (MS) is also seen. $\times 2,000$

Fig. 4 Degenerating hill type tapetal hypertrophy with primary wall. Floating tapetal cytoplasm (FT) is in the locular cavity. T: tapetal cell; DT: dilated tapetal cell; L: lipid granule. $\times 3,000$

Fig. 5 A part of a large hill type cell with very thin primary wall at the inner face of the tapetum. $\times 2,000$

Fig. 6 Hill type hyper trophy without primary wall. Arrows indicate the broken cell walls (and membranes) which separated original four cells. A microspore (MS) cut near the surface is seen on the top. N: nucleus. $\times 2,000$

Fig. 7 Hill type hyper trophy without primary wall. MS: microspore. $\times 2,000$

Fig. 8 A part of a large hill type cell without primary wall. A cavity is seen in the center. This cavity is, three dimensional, a concave from the surface of the picture (Refer to the shape in fig. 5). $\times 4,000$

Fig. 9 A presumable initiation of flame type tapetal hypertrophy. MS: microspore. $\times 4,000$

Fig. 10 Flame type tapetal hypertrophy. MS: microspore. $\times 2,000$

Fig. 11 Hill-flame type tapetal hypertrophy. $\times 2,000$

Fig. 12 A very small balloon on the surface of a tapetal cell. $\times 12,000$

Fig. 13 A small balloon and a tiny protrusion on the surface of a tapetal cell. This protrusion is a presumable figure of balloon initiation. $\times 6,000$

Fig. 14 A part of a degenerating balloon. The cell membrane disappeared. $\times 2,000$

Fig. 15 A small part of a huge balloon type hypertrophy which occupies the whole space of the locular cavity. The attached site to the original tapetal layer is seen on the right. $\times 2,000$

Fig. 16 Big balloon type hypertrophy with flame-like $\approx$ protrusions. A breaking of the cell membrane is shown by an arrow. MS: microspore. $\times 2,000$

was formed by fusing of four original tapetal cells. Three arrows in the figure indicate the broken cell walls (and membranes) which had separated the original tapetal cells. Note also the sites of the nuclei. Microspores are seen in the locular cavity in these figures.

Fig. 8 shows a part of a large hill type cell without primary wall. The cavity in the center of the figure is considered to be, three dimensional, a concave (or convex) from the surface of this photograph. Refer to the shape of the dilated cell shown in fig. 5.

Figs. 9 to 11 and 16 illustrate flame type tapetal hypertrophy. Fig. 9 shows a presumable figure of the initiation of flame type hypertrophy. Fig.

10 shows a typical flame type hypertrophy. The hypertrophy shown in fig. 11 has the features of both hill type and flame type (hill-flame type).

Figs. 12 to 16 illustrate balloon type tapetal hypertrophy. Fig. 12 shows a very small balloon on the surface of a tapetal cell (Note the magnification of the photograph). Fig. 13 shows a small balloon whose attached site to the mother cell is not on this face of the photograph, and tiny protrusion which is a presumable figure of balloon initiation.

Fig. 14 shows a part of a large balloon which is in the early phase of degeneration. The cell membrane already disappeared. Fig. 15 is a small part of a huge balloon type hypertrophy

which occupies the whole space of the locular cavity. The attached site of the baloon to the original tapetal layer is seen on the right.

Fig. 16 shows big balloon type hypertrophy which has flame-like protrusions (balloon-flame type). A microspore is included in the balloon and a broken site of the cell membrane is seen.

DISCUSSION

Tapetal hypertrophy has been classified into two major types, namely hill and balloon types, after Sakai's paper³⁾. The hypertrophy in the stage when tapetum has primary wall at the inner face is exclusively hill type (Fig. 17-1). The primary wall seems to be dissolved after the end of the meiosis.

In the stage after the disappearance of primary wall, the shape of tapetal hypertrophy is greatly variable. Besides hill and balloon types (figs. 17-2 and -3), there are flame-like figures which are denoted as flame type in this paper (figs. 17-2' and -3'). The flame type hypertrophy is not observable through optical microscope, and hence hill and balloon types have been considered to be distinguished sharply. On the basis of observations through electron microscope, however, hill and balloon types are continuous through hill-flame and balloon-flame types, though there are typical figures of hill or balloon types (fig. 17). This continuity is supported by the features of organelles in those cells.

Thus, the existence of the primary wall should

Fig. 17 A schematic illustration of the types of tapetal hypertrophy. 1. Hill type with primary wall. 2. Hill type without primary wall. 2'. Flame type (1), hill-flame type. 3. Balloon type. 3'. Flame type (2), balloon-flame type

be considered before classifying hill and balloon types.

Fig. 17 shows a newer classification of tapetal hypertrophy according to the results reported in this paper. A to D indicates the progress of the hypertrophy. Number 1 is hill type with primary wall. Fig. 1 is an example for 17-1-A, fig. 2 for 1-B, figs. 3 and 4 for 1-C, and fig. 5 for 1-D. Number 2 is hill type without primary wall. Fig. 6 is an example for 2-B, figs. 7 and 8 for 2-C. Number 3 is balloon type. Figs. 12 and 13 are examples

for 3-A and 3-B, fig. 14 for 3-C, and fig. 15 for 3-D. Number 2' and 3' are flame type; the former is hill-flame type and the latter is balloon-flame type. Fig. 9 is an example of the initiation of flame type hypertrophy. Fig. 10 is an example for 2'-B (or 2'-C), fig. 11 for 2'-C (or 2-D), and fig. 16 for 3'-D.

Besides those types described above, there are some modifications in the features of tapetal hypertrophy. One of them is the swelling of tapetal cells without the augmentation of the cytoplasm. Another is the bursting out of the cell content into locular cavity at various stages of tapetal dilatation. The cytoplasm in dilated tapetum starts to degenerate also at various stages of the dilatation.

SUMMARY

Tapetal hypertrophy was induced by cooling treatment around the young microspore stage in rice plants, and observed through electron microscope.

Observed hypertrophy was classified into following four types:

1. Hill type with primary wall
2. Hill type without primary wall
3. Flame type
4. Balloon type.

In the stage when tapetum has primary wall at the inner face, induced hypertrophy was exclusively hill type. After the disappearance of primary wall, the other three types (hill without primary wall, flame and balloon types) were observed. These three types were continuous in shape and in cytoplasmic ultrastructure, through intermediate hill-flame and balloon-flame types.

LITERATURE CITED

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〔和 文 摘 要〕

イネの小孢子初期冷温処理による雄性不稔

第12報 微細構造にもとづくタペート肥大の分類

西 山 岩 男

(農林省北海道農業試験場)

小孢子初期前後に冷温処理をおこなつてタペート肥大をおこさせ、それを電子顕微鏡で観察した。観察された肥大はつぎの4型に分類された：

1. 1次壁のある丘状肥大	3. 炎状肥大
2. 1次壁のない丘状肥大	4. 風船状肥大

タペート細胞の葯腔側に1次壁のある時期においては、肥大はすべて丘状であつた。この1次壁が消失して以後に他の3型(1次壁のない丘状、炎状、風船状)が観察された。これら3型は、丘—炎状および風船—炎状などの中間型を介して連続的であり、細胞質の微細構造も共通している。