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

VII. Electron microscopical observations on tapetal cells dilated by the cooling treatment**

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In the preceding paper¹, the author reported electron microscopical observations on normal tapetal cells at the critical stage to coolness. This paper deals with observations on dilated and fused tapetal cells induced by cooling treatment at the critical stage.

MATERIALS and METHODS

A paddy rice variety, Norin 20, was cultured and cooled in artificial light rooms of the phytotron. The cooling treatments were made at 12°C for 3 days around the meiotic and young microspore stages. After the treatments, anthers were taken from young spikelets of about 3.5 to 4.0 millimeters in length. These anthers were from the second division of meiosis to the first contraction phase of microspores. Processes from fixation to sectioning and staining were performed as described in the preceding paper².

RESULTS

Fig. 1 shows a large dilated and fused tapetal cell at the second division of the meiosis. This cell consists of five original cells in the plane of this photograph. The sites of four broken walls are shown by arrows. Nuclei are shrunk and stained denser than the normal appearance (refer to fig. 4 and the preceding paper²). Endoplasmic reticula are much swollen.

* The title of this series was revised since the fifth report. The former title was "Male sterility caused by cooling treatment at the meiotic stage in rice plants."
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Fig. 2 is a magnified part of fig. 1. Proplastids, mitochondria, small vacuoles and callose walls seem not to be injured morphologically. A vestige of a broken intertapetal cell wall is seen. At the broken site, the cell membranes of two original cells are connected (see also figs. 6 and 7). Golgi bodies are often gathered closely in tapetal cells (fig. 3). This is also one of normal features.

Fig. 4 shows two slightly dilated cells at the nascent microspore phase. The cell wall separating them are broken. In the vicinity of the breakage, cytoplasm is much diluted. Nuclei and endoplasmic reticula, however, are still normal. This figure might show the first morphological change leading to the huge dilated cell. An advanced feature of the dilated cell is shown in fig. 5. The endoplasmic reticula are furthermore swollen and the cytoplasm are condensed. Nevertheless, proplastids and mitochondria are still not deformed.

Figs. 6 and 7 are the photographs of parts of large dilated cells at the nascent microspore phase. Endoplasmic reticula are slightly swollen, but proplastids, mitochondria, vacuoles and Golgi bodies seem to be normal. In fig. 6, orbicules are seen under the callose wall. Note also broken cell walls. A huge case of the dilatation is shown in fig. 8. This cell has enlarged more than half of the locular cavity.

The density of ribosomes and polysomes, and the number of ribosome unit in a polysome do not show any perceptible change in those early stages of hypertrophy (fig. 9) except the case of fig. 5, in which ribosomes are not clear.
Fig. 1. A dilated and fused tapetal cell at the second division of the meiosis. Arrows show the sites of four broken walls of original cells. Nuclei (N) are deformed and endoplasmic reticula (ER) are swollen. × 5,333.

Fig. 2. A magnified part of fig. 1. Proplastids (P), mitochondria (M), vacuoles (V) and callose wall (W) show normal appearances. A fragment of a broken intertapetal cell wall is shown by an arrow. Note the connected cell membranes of two original cells. × 16,000.
Fig. 3. Gathered Golgi bodies (G). Six or seven bodies are seen in the plane of this photograph. $\times 24,000$.

Fig. 4. Two cells which are slightly dilated and joined by a pore of the separating wall. This is considered to be the initial process of hypertrophy. $\times 5,333$.

Fig. 5. An advanced feature of the dilated cell. Endoplasmic reticula are further swollen and cytoplasm are condensed. $\times 5,333$. 
Fig. 6. A magnified part of a dilated cell at the nascent microspore phase. Orbi-
cules (O) under the callose wall seems unaffected. Proplastids (P), mito-
chondria (M), vacuoles (V), swollen endoplasmic reticula (ER) and a bro-
ken cell wall (arrows) are seen. × 16,000.

Fig. 7. A magnified part of a dilated cell at the nascent microspore phase. Pro-
plastids (P) including electron-dense granules, mitochondria (M), vacuoles
(V), Golgi bodies (G), endoplasmic reticula (ER) and a broken cell wall
(arrow) at the side of transitory cells are seen. × 16,000.
DISCUSSION

Sakai reported that tapetal hypertrophy occurred from the prophase of the first meiotic division in rice plants subjected to the cool weather in the summer of 1941\(^a\), but did not occur until the nascent microspore phase in experimentally cooled rice plants\(^b\). He\(^b\) considered that the hypertrophy in 1941 might not have occurred earlier than those of the experiments, but rather the development of the pollen mother cells might have been delayed. While, our present paper showed that tapetal hypertrophy was induced experimentally at the second division of the meiosis. In this case, the pollen developmental stages did not delay on the basis of palea length\(^b\). (Palea length is a good indicator of pollen development and the paleae are not injured by cooling treatment though the elongation rate slowed down during the treatment.\(^b\))

Sakai\(^b\) also described that there were two kinds of tapetal hypertrophy in rice plants cooled at the meiotic stage: one was the balloon type and the other was the hill type. According
Table 1. Normal and abnormal organelles in dilated tapetal cells.

<table>
<thead>
<tr>
<th>Normal organelles</th>
<th>Abnormal organelles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proplastids with electron dense granules</td>
<td>Nuclei………………….deformation</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Endoplasmic reticula…………………swelling</td>
</tr>
<tr>
<td>Golgi bodies with vesicles</td>
<td>Intertapetal walls and cell membranes</td>
</tr>
<tr>
<td>Vacuoles</td>
<td>……………………breakage</td>
</tr>
<tr>
<td>Orbicules</td>
<td>Cell components*………………….proliferation</td>
</tr>
<tr>
<td>Callose walls</td>
<td></td>
</tr>
<tr>
<td>Ribosomes and polysomes</td>
<td></td>
</tr>
</tbody>
</table>

* Cell components without nuclei and endoplasmic reticula are normal in shape, but have proliferated abnormally.

to our observations, the greater part of hypertrophy was the hill type. The author has not obtained any balloon type of pictures in the electron microscopy. As far as concerning the hill type, the large cell is clearly made of, in the transverse section, several original tapetal cells which fused mutually by the breakage of cell walls and membranes.

The nuclei of dilated cells are deformed and any dividing figures of them have not been found. The endoplasmic reticula are swollen. Other organelles, however, not only seem uninjured but also have proliferated, because dilated cells are still rich in cytoplasmic elements in spite of their enlargements in volume. These morphological features of dilated tapetal cells are summarized in Table 1. Generally, those features do not depend on the phase when the dilatation initiated. But minor differences can be pointed. For example, orbicules are seen in figs. 6 and 8 but have not yet appeared in fig. 2.

The swelling of endoplasmic reticulum might be due to the cessation of their function, which is to produce substances available only to pollen, such as sporopollenin. Cytoplasmic elements except endoplasmic reticula and nuclei might have proliferated by using nutritive substances which were to be supplied to pollen mother cells or microspores. It is not yet clear, however, whether cool injury in tapetum or in microspores or in both leads to the inhibition of nutrient translocation. Fig. 3 might show the earliest morphological change in tapetal hypertrophy. If it is the fact, the cause of the hypertrophy can be attributed to an increase in turgor pressure within the cells. Increases in cytoplasmic components and the deformation of nuclei and endoplasmic reticula might follow the wall breakage by the increased turgor pressure.

**SUMMARY**

Tapetal hypertrophy was induced by cooling treatment at the young microspore stage in rice plants. The dilated and fused tapetal cells were observed electron-microscopically.

In those cells, nuclei were deformed, endoplasmic reticula were swollen and walls (and cell membranes) which had separated original tapetal cells were broken. Cell components other than nuclei and endoplasmic reticula seemed to be morphologically normal, but they proliferated much.

**ACKNOWLEDGEMENT**

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**LITERATURE CITED**

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[和文摘要]

イネの小胞子初期冷却処理による雄性不稔
第7報 冷却処理により肥大したタペット細胞の電子顕微鏡的観察

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小胞子初期ころに冷却処理をおこさせ、それを電子顕微鏡により観察した。肥大細胞は（横断切片の面において）数個のものとのタペット細胞が融合してできたもので、核は菱様変形し、小胞体は膨潤し、やぶれた細胞壁（および細胞膜）は斑痕として観察された。その他の細胞要素は形態的に正常であったが、肥大にともなって相当に増殖していた。