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

VI. Electron microscopical observations on normal tapetal cells at the critical stage**

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Sakai found an abnormal hypertrophy of tapetal cells in rice plants cooled at the meiotic stage⁶⁾. He considered that this hypertrophy is the most important cause of the sterility induced by cool weather^{7,8)}. Although the frequency of tapetal hypertrophy was shown not to be sufficient for an important cause of the sterility⁵⁾, the hypertrophy seems to be closely related with incipient processes leading to sterility.

In this paper, the results of electron microscopical observations of normal tapetal cells at the young microspore stage are reported. The observations on dilated tapetal cells will be dealt with in the succeeding paper⁴⁾.

MATERIALS AND METHODS

A paddy rice variety, Norin 20, was cultured in an artificial light room of the phytotron through its entire life. The anthers were excised from young spikelets at the end of meiosis to the young microspore stage. Both ends of the excised anthers were cut off in 2.5 per cent glutaraldehyde solution (buffered with 0.1 M phosphate at pH 7.0). The cut materials were fixed at room temperature for 60 to 120 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 per cent osmium tet-

roxide (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 6 per cent uranyl acetate for 60 minutes and with one-tenth diluted Millonig's lead solution for 15 minutes.

RESULTS

Fig. 1 shows tapetal cells and parts of two pollen mother cells at the second division of meiosis. Tapetal cells do not have any large vacuole and are rich in cytoplasm. The large vacuoles of transitory cells are seen at the bottom left (see also fig. 3). Fig. 2 is a larger magnification of a tapetal cell at the same stage as fig. 1. The nucleus is ovoid and the rough-surface endoplasmic reticula are beginning to develop. Proplastids have undeveloped thylacoids and often include electron-dense granules which might be glycogen-like polysaccharides. Some of the mitochondria are swollen. The vacuoles are small and not so many. The cell wall is very thin at the site of two tapetal cell contact. In the part facing the locular cavity it is a little thicker and consists of callose. Either parts of the cell wall must be weak mechanically as compared to the thicker cellulose wall of ordinary plant cells. At this stage, tapetal cells are convex to the locular cavity.

Fig. 3 shows tapetal cells at the nascent microspore phase. The degradation of the cells is already beginning. The cavity side of the cell is flat and the intertapetal cell contacts are broken at some parts. Fully developed endoplasmic reticula are seen in the leftmost cell.

* 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. 1. Tapetal cells (T) and parts of two pollen mother cells (PMC) at the second division of the meiosis. $\times 4,000$.

Fig. 2. A tapetal cell at the second division of the meiosis. A nucleus (N), proplastids (P), mitochondria (M), endoplasmic reticula (ER), vacuoles (V), and walls (W) are seen. $\times 16,000$.

Fig. 3. Tapetal cells at the nascent microspore phase. Fully developed endoplasmic reticula (ER) are seen. The intertapetal cell contacts are broken at some parts (arrows). $\times 6,000$.

Fig. 4. A part of a tapetal cell at the nascent microspore phase. Orbicules (O) are between the callose wall and the cell membrane. A part of a nucleus (N), proplastids (P), mitochondria (M), an endoplasmic reticulum (ER) and Golgi bodies (G) are also seen. $\times 16,000$.

Fig. 5. Vesicle formation from the terminals of Golgi cisternae. $\times 48,000$.

Fig. 6. A part of a tapetal cell at the first recovering phase of microspores. Orbicules (O) with spinules are exposed to the locular cavity. A part of a nucleus (N), proplastids (P), mitochondria (M), an endoplasmic reticulum (ER) and polysomes (arrows) are seen. $\times 24,000$.

endoplasmic reticulum and polysomes are seen.

DISCUSSION

We suggested in a previous paper that the suppression of pollen maturation might be the main cause to the sterility induced by cooling treatment at the so-called meiotic stage in rice plants²⁾. This immaturation became perceptible about three days after the cooling treatment³⁾. The causal links from coolness to the immaturation have not been clarified as

yet. While tapetal hypertrophy is one of the earliest symptoms of the injury caused by cool temperature at the critical stage. The frequency of hypertrophy was not sufficient to be an important cause to sterility¹⁾. Therefore, the hypertrophy was considered to be only a small by-pass. However, the hypertrophy and the pollen immaturation might have their common cause, that is, the preceding abnormality or abnormalities induced directly by coolness (fig. 7). If it is the case, these earlier abnormalities will be able to be discovered by tracing the processes back to the hypertrophy. This is why we commenced these studies on the ultrastructure of tapetal hypertrophy.

At the first recovering phase of microspore, tapetal cells become concave to the locular cavity. Fig. 6 shows a part of the cell at this phase. The callose wall dissolved away and orbicules are exposed. Electron-dense spinules are growing on the surface of the orbicules. Nucleus, proplastids, mitochondria, rough-surface

Fig. 4 is a magnified part of a tapetal cell at the same stage as fig. 3. Proplastids became larger and less dense as compared with those of the meiotic stage. Many orbicules appeared under the callose wall. Nucleus, mitochondria and Golgi bodies are also seen. Vesicle formation from the terminals of Golgi cisternae are shown in fig. 5.

Fig. 7. Causal sequence from cool temperature to sterility.

The tapetum is a secretory tissue. The cells do not have any large vacuoles and are rich in cytoplasm. Endoplasmic reticula develop from the second division of the meiosis and become large masses at the young microspore stage. Proplastids including electron-dense granules grow during the period. Nuclei with nucleoli, mitochondria, vesicle-forming Golgi bodies, vacuoles, ribosomes and polysomes do not seem to change. The walls are generally very thin and weak. They are often hardly perceptible at intertapetal sites. At sites facing the locular cavity, they initially consist of callose and later the callose fades away exposing cell membranes. This weakness should be related to the degrading fate of the cells. Another prominent feature of tapetal cells are orbicules. They are born as proorbicular bodies under cell membranes facing to the cavity at the end of the meiosis and become orbicules after penetrating the membranes⁸⁾. Electron-dense spinules, may be made of sporopollenin⁹⁾, grow on the surface of the orbicules.

These features of tapetal cells will be discussed in comparison with those of dilated cells in the succeeding paper⁴⁾.

SUMMARY

Normal tapetal cells were observed electron microscopically at the critical stage to coolness. The cells are rich in cytoplasm and do not have any large vacuoles. Endoplasmic reticula develop from the second division of the meiosis and became large masses at the young microspore stage. Cell walls initially consist of callose at the sites facing the locular cavity and later the callose dissolved away exposing cell membranes. At the sites of two tapetal cell contact, the

walls are very thin. Orbicules are seen under callose wall at the nascent microspore phase and exposed to the cavity with the disappearance of callose at the first recovering phase of microspores. Electron-dense spinules grow on the surfaces of the exposed orbicules. These morphological features coincide with the secretory nature and the degrading fate of the cells.

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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6報 冷温感受性期における正常なタペート細胞の電子顕微鏡的観察

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冷温にたいしてもつとも感受性のたかい小孢子初期ころのタペート細胞を電子顕微鏡により観察した。タペート細胞は細胞質が充実しており、おおきな液胞をもたない。粗面小胞体が減数分裂のおわりごろから発達しはじめ、小孢子初期にはおおきなカタマリになる。タペート細胞間の細胞壁は非常にうすく、ほとんど細胞膜のみからなっている。葯腔側の細胞壁はカロースでできており、のちにカロースがとけて細胞膜が露出する。新生小孢子期にこのカロース壁の下に球状体が生じ、カロース壁がとけさると球状体も露出する。そのご電子密度のたかい突起が、この球状体の表面に生長していく。以上のような形態的特長は、タペート細胞の分泌的性質およびまもなくこわれていく運命とよく符合している。