Brief Notes

Pathological Cytology of Tobacco Leaf Infected with Tobacco Mosaic Virus III. By CHIAKI MATSUI.
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The idea that chloroplasts are the sites of virus formation is not new. Since Kausche and Ruska (4) published an electron micrograph showing filamentous particles of tobacco mosaic virus associated with chloroplast fragments, the speculation that virus particles are formed within chloroplasts and released from them into the surrounding cytoplasm, has been widely supported by successive investigators (1, 5, 8, 10). Since these investigations were done at a time when fixation methods of tissue cells were not entirely satisfactory, it may be desirable to repeat these investigations by using more effective techniques.

In the present study, the intracellular localization of virus particles within host cells was investigated by the electron microscopy of thin sections of young tobacco leaf (*Nicotiana tabacum* L. var. Xanthi) showing slight symptoms of systemic infection with tobacco mosaic virus of common strain. The specimens were fixed at 2°C. for 2 hours in a solution of 1 per cent osmium tetroxide buffered with veronal acetate to pH 7.4. These were subsequently dehydrated and embedded in butyl methacrylate. Thin sections were cut with a JUM-4 ultramicrotome (Japan Electron Optics Laboratory Co., Ltd.) and were examined in a JEM-5G electron microscope (Japan Electron Optics Laboratory Co., Ltd.) without removal of the embedding medium.

In Fig. 1, the chloroplasts of slightly diseased leaf cells are built up of regularly organized lamellae and grana embedded in a granular matrix. These structural characteristics correspond exactly to those found in the chloroplasts of healthy tobacco leaves (2, 6) and of some other higher plants (3, 9). The fibrous masses of virus rod particles can be easily recognized within the cytoplasm. In these fibrous masses, the individual virus rod particles are generally disposed parallel to one another. Although the virus rod particles come close to the surface of the chloroplasts, they were never found within the latter. A cytoplasmic region showing more details than Fig. 1 is given in Fig. 2. In this electron micrograph, the virus rod particles rather seem to be associated with endoplasmic reticulum. According to the author's observations (6), the disintegration of chloroplasts within slightly diseased leaf cells were previously observed after FAA fixation of tissue cells or after removal of embedding medium (1, 8, 10). As illustrated in Fig. 3, the disorganization of lamellae and grana of chloroplasts within the heavily diseased leaf cells are actually observed even when these cells are fixed in buffered 1 per cent osmium tetroxide solution, while the full disintegration of chloroplasts illustrated in previous electron micrographs (1, 8, 10) are difficult to find, and the virus rod particles or virus-like particles are never encountered within them. Presumably the disorganization of the chloroplasts is a secondary event, not directly related to the virus formation in the plastids.

In the light of the present evidence, the possibility of actual formation of tobacco mosaic virus rod particles within chloroplasts seems remote and the intimate association of virus rod particles with chloroplasts emphasized in previous electron microscope studies (1, 5, 8, 10) could be interpreted as secondarily established. The relationship between the virus rod particles and young plastids will be discussed in a later paper (7).

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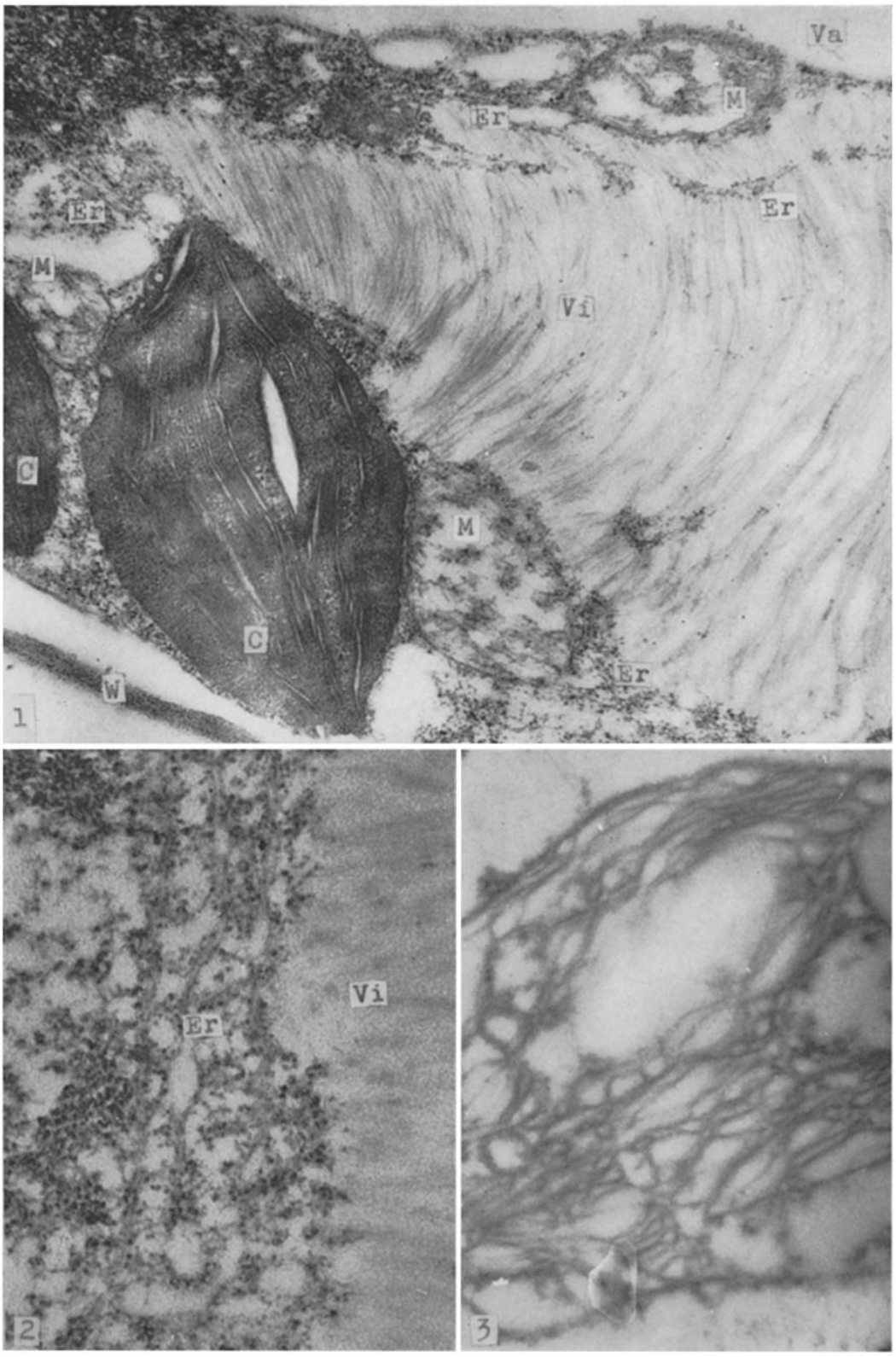
EXPLANATION OF PLATE 419

FIG. 1. Small field at the periphery of a spongy tissue cell infected with tobacco mosaic virus, showing a general view of the internal organization of the cell. The cytoplasm existing between vacuole (*Va*) and cell wall (*W*) contains the chloroplasts (*C*), mitochondria (*M*), endoplasmic reticulum (*Er*), and virus rod particles (*Vi*). Note the absence of virus rod particles within chloroplasts, mitochondria, and vacuoles. × 52,000.

FIG. 2. A portion of cytoplasm containing numerous profiles of the endoplasmic reticulum (*Er*) and virus rod particles. × 66,000.

FIG. 3. A chloroplast within heavily diseased leaf cell. Note the disorder of grana and lamellae, and the absence of virus rod particles within the chloroplast. × 46,000.



(Matsui: Tobacco mosaic virus III)