Electron Microscopy of Satsuma Dwarf Virus in Host Cells

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SATSUMA DWARF virus (SDV) has been mechanically transmitted to several herbaceous plants and from them back to satsuma orange seedlings (1, 2, 6). In 1964, the virus was purified and shown to be spherical in shape and about 26 nm in diameter (5). The study to be reported here was initiated to determine whether SDV virus particles can be observed in infected cells by electron microscopy and whether cellular changes associated with the infection can be detected.

Materials and Methods

The SDV isolate used in this study was obtained from Dr. K. Kishi. Ultrathin sections were prepared from young leaves of satsuma orange and several kinds of herbaceous plants infected with SDV, including the following: sesame, kidney bean, cowpea, *Nicotiana clevelandii* Gray, and *Chenopodium quinoa* Willd. Small portions of infected leaves were fixed with glutaraldehyde, postfixed with osmium tetroxide, and embedded in epon. The sections were double stained with uranium acetate and lead nitrate. A Hitachi 11-B electron microscope was used.

Results

Examination of sections of infected leaves under an electron microscope revealed intracellular inclusions. They were shown to consist of vesicles in groups and to occur in a cytoplasmic matrix (Fig. 1). No viruslike particles were seen within them. Because of their small size, the inclusions were difficult to detect when epidermal strips stained with Giemsa solution or phloxine were examined under a light microscope.

In the vicinity of the vesicular inclusions, viruslike particles were scattered in the cytoplasm and enclosed in tubules, which were bounded by a double membrane (Fig. 2). The viruslike particles corresponded in shape and size—approximately 20–22 nm in diameter—to infective virus particles purified from diseased plants. They are presumed to be the particles of SDV and will be referred to hereafter as virus particles. When scattered in the cytoplasm, the virus particles were not easily distinguishable from ribosomes because of the similarity in their sizes.

The tubular structures were found frequently penetrating plasmodes-

mata—or occasionally in the space between the cell wall and the cytoplasmic membrane in the vicinity of the plasmodesmata—but not within the cytoplasm (Fig. 3). At the end of the tubular structure, a portion of the cytoplasm surrounded by the cytoplasmic membrane had protruded into the vacuole (Fig. 4). The ends of the tubular structure were frequently found to be open into the cytoplasm. The tubular structures contained arrays of spherical virus particles. Figure 5, which is at a higher magnification than Figure 4, clearly shows that the tubular structure has a double membrane, which is presumed to be connected with a cytoplasmic membrane. The diameter of the outer tube is 55–60 nm and that of the inner tube is 35–45 nm.

Virus particles were also observed in lattice structures, or in crystalline arrays in the cytoplasm, or in a central vacuole (Figs. 6, 7). No virus particles were observed in the nuclei, the chloroplasts, or the mitochondria of the cells of the various plants examined.

Discussion

Intracellular forms of virus and certain ultrastructural changes in cells of the various plants examined were detected by means of electron microscopy. They include: 1. vesicular inclusions in a cytoplasmic ma-

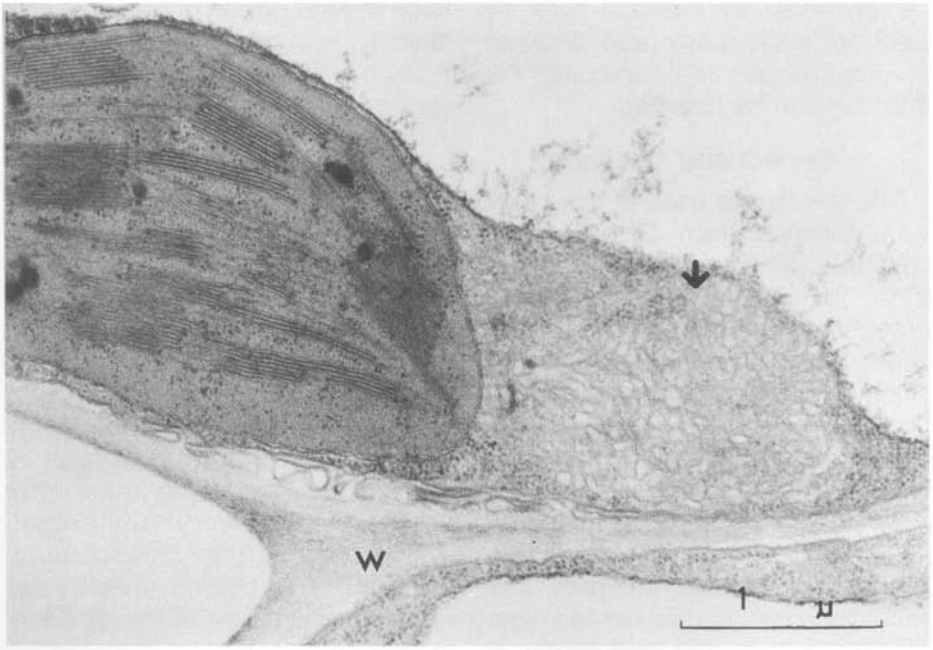

FIGURE 1. *Electron micrograph of an area of the cytoplasm of a cell from a sesame leaf inoculated 20 days previously with SDV. Note the intracellular inclusion (arrow) consisting of vesicles in groups. W, cell wall.*

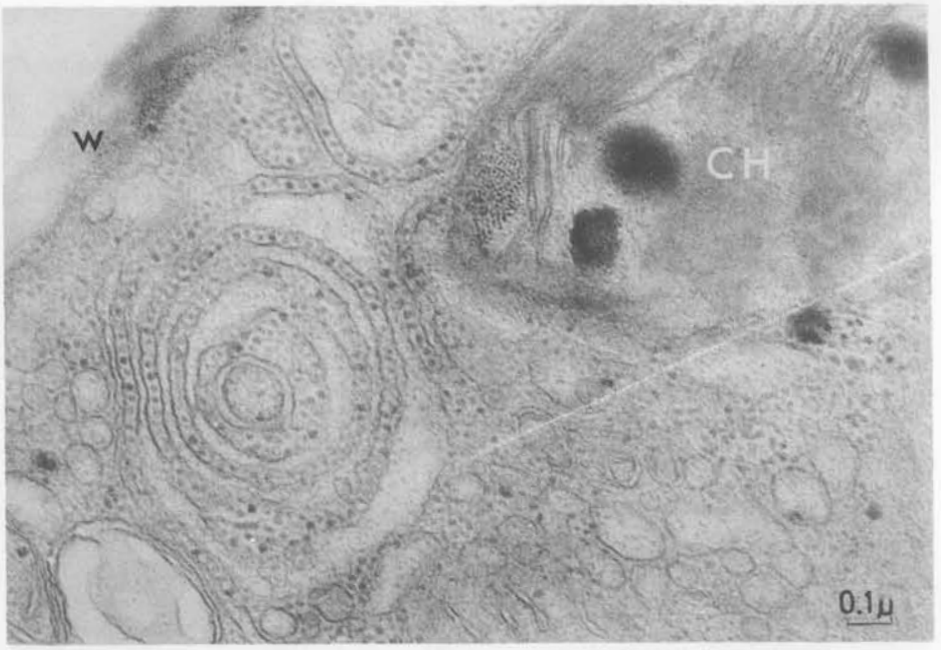

FIGURE 2. *Electron micrograph of a portion of a cell, cytoplasmic area, from a sesame leaf systemically infected with SDV. Note the virus particles scattered in the cytoplasm and generally enclosed in a tubular structure bounded by a double membrane. Phytoferritin can be seen within a chloroplast. CH, chloroplast; w, cell wall.*

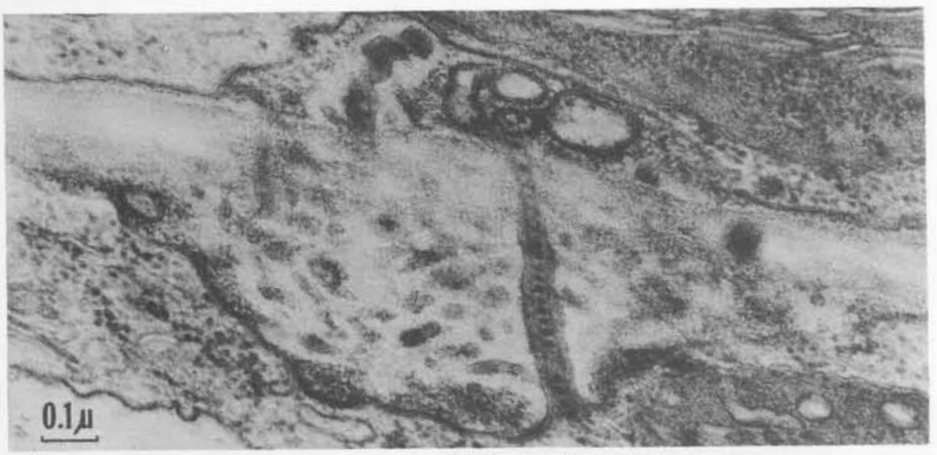

FIGURE 3. *Electron micrograph of longitudinal section through a plasmodesma in a cell of a systemically infected leaf of a sesame plant inoculated with SDV 64 days previously. Note the tubular structure, containing an array of virus particles, penetrating the plasmodesma. One end of the tubule seems to open into the cytoplasm in the lower cell.*

trix; 2. modified plasmodesmata, tubular structures containing virus particles, the tubule sometimes being enclosed in a portion of the cytoplasm that has protruded into the vacuole; 3. virus particles scattered in the cytoplasm; and 4. virus particles in lattice structures within the cytoplasm or the vacuole.

Milne (4) reported the occurrence of rounded and partly crystalline aggregates of virus particles in *Chenopodium amaranticolor* leaves systemically infected with cowpea mosaic virus. The crystalline arrays of SDV do not resemble the aggregates of cowpea mosaic virus.

Kitajima and Lauritis (3) recently reported the occurrence of modified plasmodesmata in zinnia leaves in-

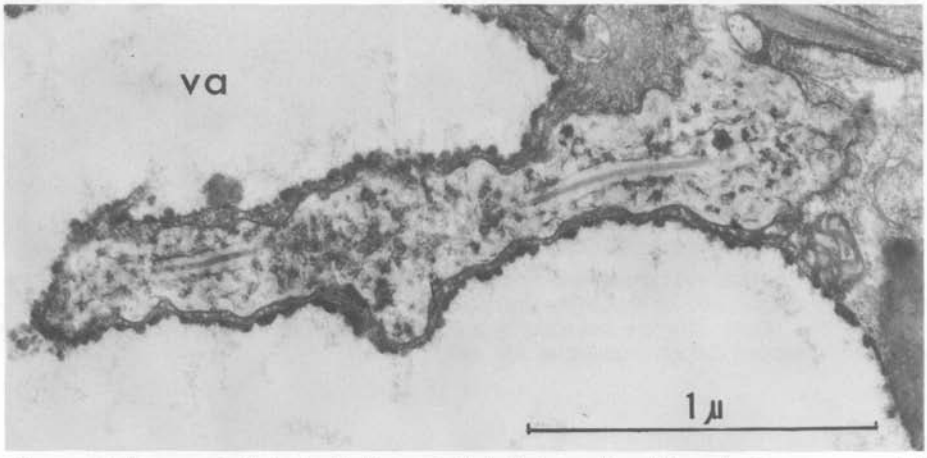

FIGURE 4. *An area similar to that in Figure 3. Note that a portion of the cytoplasm surrounded by the cytoplasmic membrane has protruded into the vacuole. Va, vacuole.*

FIGURE 5. *A higher magnification of the tubular structure in Figure 4. Note the double membrane of the tubular structure.*

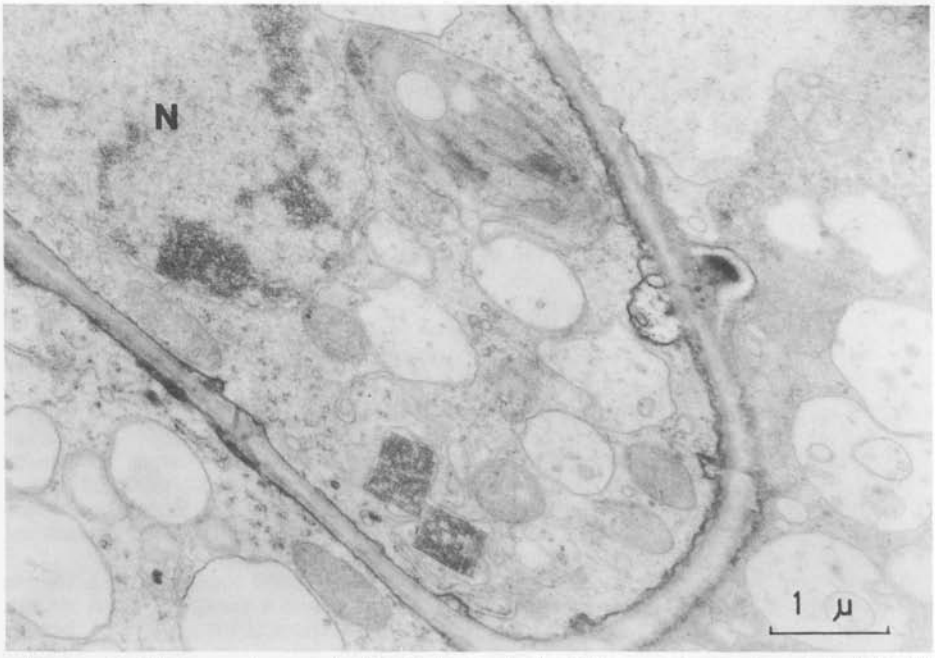

FIGURE 6. *Electron micrograph of cells from a leaf of* Chenopodium quinoa *infected with SDV. Note virus particles in lattice structures. Penetration of the cytoplasmic membrane can also be seen.*

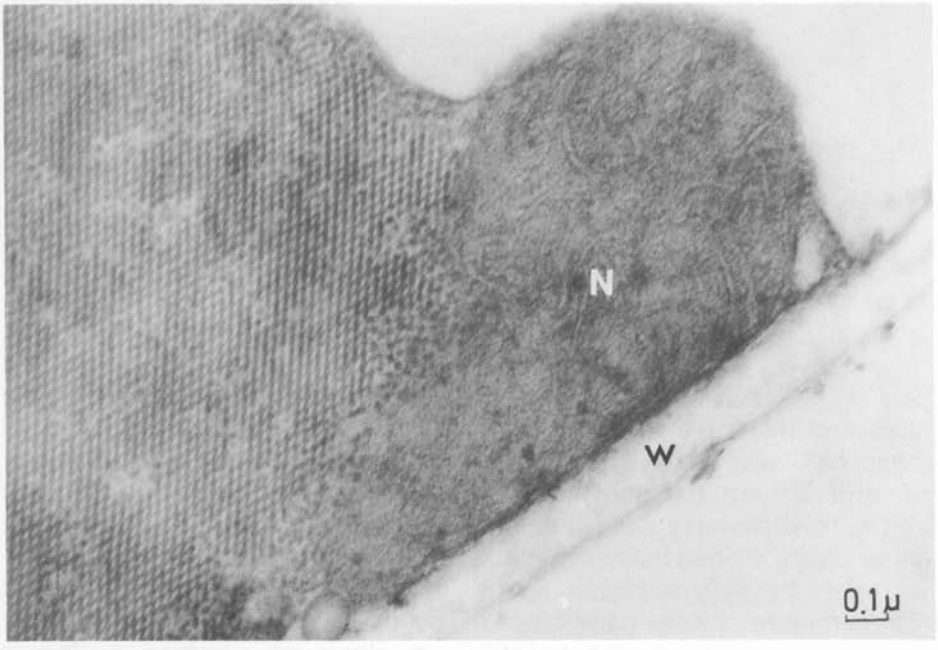

FIGURE 7. *Lattice structure similar to that in Figure 6—at a higher magnification—from a cell of a leaf of* Nicotiana clevelandii *systemically infected with SDV. N., nucleus; w, cell wall.*

fected with dahlia mosaic virus—modified plasmodesmata that are similar to those observed in the case of SDV. Tubular structures and conspicuous swelling of the cytoplasmic membrane in the vicinity of plasmodesmata are characteristic of plants infected with SDV.

The virus particles, as well as the ultrastructural changes, were abundant in the cells of the infected herbaceous plants tested but were scarce in the cells of infected satsuma orange leaves. The difference may correspond with the lower concentration of virus in the cells of satsuma orange.

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