Nuclear Ultrastructural Changes and Aggregates of Viruslike Particles in Mungbean Cells Affected by Mungbean Yellow Mosaic Disease

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ABSTRACT

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Yellow mosaic disease of mungbean in Thailand was caused by a whitefly-transmitted agent, presumably a virus. Ultrastructural changes similar to those previously reported for whitefly-transmitted viruses are associated with the disease. The viruslike particles (VLPs) associated with the disease were isometric, about 15–20 nm in diameter, and often formed loose aggregates that sometimes almost filled the total nuclear volume of infected phloem cells. Mungbean infected by whitefly transmission or by grafting had hypertrophied nucleoli, aggregates of VLPs, and fibrillar bodies in the nuclei of phloem cells as early as 2 days before symptom appearance. In vacuoles or lumens of the partially or fully differentiated infected sieve elements, VLPs occasionally formed aggregates having a double cylindrical arrangement of particles. No VLPs were detected in tissues other than the phloem of infected plants or in any tissues of comparable healthy plants.

Additional key word: geminivirus.

A group of plant viruses has recently been recognized which has a common characteristic of paired or geminate particles with an unusually small size (15–20 nm in diameter) for the individual particles (2,4,6,7,12,14). The geminate particles are found not only in purified preparations, but also in situ (8). In addition, the nucleic acid of several viruses of this group is single-stranded DNA (5,7). These properties are unique among previously known plant viruses and the name geminivirus was proposed (7).

Geminiviruses are transmitted either by whiteflies or leafhoppers in nature. The whitefly-transmitted group includes members such as bean golden mosaic virus (BGMV) (6), Euphorbia mosaic virus (EMV) (1), and tobacco leaf curl virus (TLCV) (14); the leafhopper-transmitted group includes maize streak virus (MSV) (2), beet curly top virus (BCTV) (12), and chloris striate mosaic virus (CSMV) (4).

In 1977, an outbreak of mungbean yellow mosaic disease occurred in northern Thailand. Our preliminary tests indicated that the causal agent of the disease was transmitted by the cotton whitefly (*Bemisia tabaci* Genn.). Infected plants had bright yellow mosaic symptoms and produced few pods. The transmissibility of the causal agent and the symptoms it induced in infected plants were similar to those reported for mungbean infected with mungbean yellow mosaic virus in India (13). Although neither the causal agent of the disease in Thailand nor in India has been studied ultrastructurally, we presumed that both belong to the geminivirus group because of their transmission by whiteflies and the symptoms induced in infected plants.

We have conducted ultrastructural studies on mungbean infected with mungbean yellow mosaic disease (MYMD) which occurred in Thailand and report for the first time the association of small VLPs in the nuclei of cells from infected plants. In addition, we also report an unprecedented pattern of aggregation of VLPs that had a double cylindrical arrangement in the sieve elements of infected mungbean plants.

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MATERIALS AND METHODS

Mungbean (*Vigna radiata* [L.] Wilcz.) plants were infected with MYMD agent transmitted either by whitefly or by grafting. For whitefly transmission, cotton whiteflies (*B. tabaci*) were allowed an access period of 48 hr each for acquisition and inoculation feeding. For inoculation by grafting, healthy plants were implanted with tissues from plants infected by whitefly transmission. After inoculation, plants were maintained in an insect-proof cage at Bangkok (14° N latitude) or in glass cabinets at the Institute for Plant Virus Research in Japan (36° N latitude). The cabinets were set at 32 C day and 28 C night, and 90% RH. Supplementary lighting to give a 14-hr photoperiod was provided by two 40 W fluorescent lamps located at about 1.5 m above the cabinet floor.

Leaf samples for electron microscopy were collected from inoculated plants 5, 7, 9, 11, 13, 15, and 18 days after inoculation and processed by previously reported methods (9), except that a low-viscosity epoxy resin (16) was used for embedding. Sections were cut with a glass knife in a Porter-Blum Model MT 2B ultramicrotome and were double-stained with uranyl acetate and lead citrate before observation with a Hitachi Model H300 or H500 electron microscope previously calibrated with carbon gratings for magnification readouts. The sizes of VLPs were measured directly on the negatives with the aid of a magnifying lens.

Leaf samples from noninoculated mungbean plants at comparable age were similarly processed and served as controls.

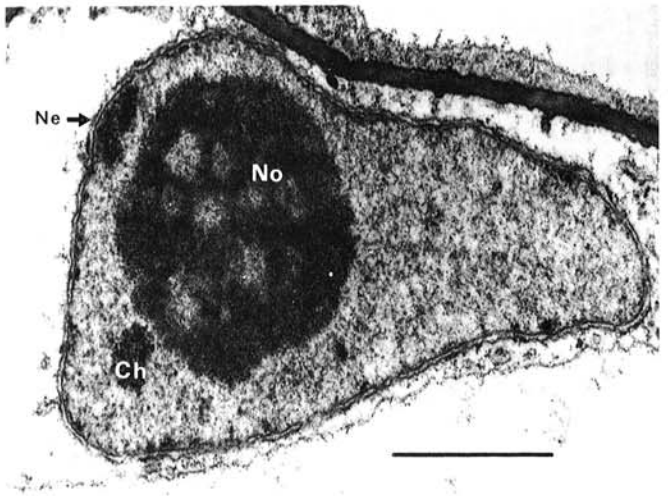

Fig. 1. Hypertrophied nucleolus (No) of a mungbean yellow mosaic disease-affected phloem parenchyma cell of a mungbean plant 2 days before symptom appearance. Ne, nuclear envelope; Ch, chromatin. Scale bar = 1,000 nm.

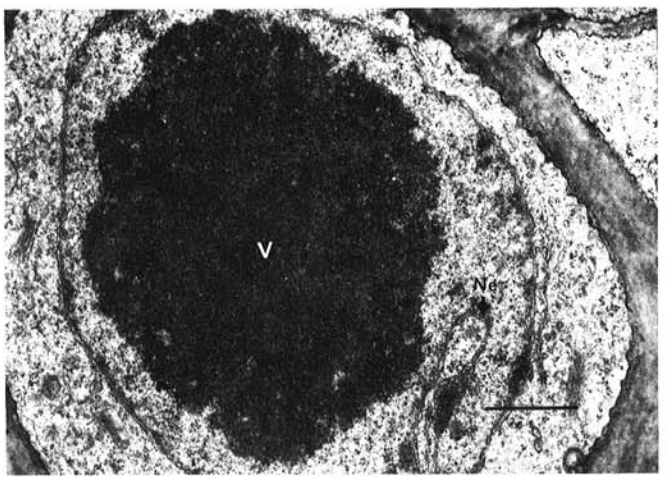

Fig. 2. Aggregate of viruslike particles (V) occupying almost the total nuclear volume of an infected sieve element of mungbean affected by mungbean yellow mosaic disease. Ne, nuclear envelope. Scale bar = 1,000 nm.

RESULTS

The first ultrastructural changes observed were in phloem tissues sampled 7 days after inoculation (2 days prior to symptom appearance). The nucleoli of some phloem cells from such symptomless tissue were hypertrophied (Fig. 1). Loose aggregates of VLPs also were observed in the nucleus. The aggregates varied in size and shape, but usually were round and sometimes occupied almost the total nuclear volume (Fig. 2). Aggregates of VLPs were more frequently observed in the nucleus as the time after inoculation progressed. These aggregates were found in nuclei whether the nucleoli were present or absent. In nuclei containing nucleoli and aggregates of VLPs, the size of the nucleolus was usually smaller than that of the aggregate (Fig. 3a) although occasionally both were of the same size (Fig. 3b). The aggregates were highly electron-dense and the outlines of small VLPs were evident, in comparison to the ill-defined contents of the nucleolus (Fig. 3b). Fibrillar bodies (usually one or two per nucleus) with the shape of either solid circles (Fig. 3b) or rings (Fig. 3c), depending upon the orientation of sectioning, were occasionally observed along the edge of the aggregates of VLPs or scattered in the nucleoplasm (Fig. 3d). The diameter of the VLPs was 15–20 nm.

Viruslike particles were observed in the vacuoles or lumens of partially or fully differentiated sieve elements; the particles were either scattered in the sieve tube (Fig. 4) or arranged to give loose or paracrystalline aggregates (Fig. 5). In addition, aggregates of VLPs having a double cylindrical arrangement were observed (Fig. 6). The double cylindrical aggregates consisted of rows of VLPs with indefinite numbers of particles to give small cylinders, each having five rows of particles (Fig. 6, inset). About seven or eight of these small cylinders were in turn arranged to form a large cylinder. The number of the small cylinders giving rise to each large cylinder could not be precisely determined since the side-by-side arrangement of the neighboring rows of particles were slightly twisted, so that some small cylinders were cut tangentially in the plane perpendicular to the cylinder axis.

Although hypertrophied nucleoli and VLPs and their aggregates were present in tissues sampled 2 days, but not 4 days, before symptom appearance, they were more frequently observed in tissues sampled several days after symptom appearance. Tissues sampled 7 and 10 days after symptom appearance often were necrotic and VLPs were rarely observed. No differences in ultrastructural changes were detected between tissues infected by whitefly transmission or grafting. No ultrastructural changes or VLPs were observed in comparable healthy tissues.

DISCUSSION

Our findings of ultrastructural changes and VLPs in phloem cells of mungbean plants are the first direct evidence indicating that the yellow mosaic disease of mungbean in Thailand may be caused by a virus. The ultrastructural abnormalities in infected phloem cell nuclei (ie, hypertrophied nucleoli, fibrillar bodies, and aggregates of VLPs) were similar to those reported for several other diseases induced by whitefly-transmitted geminiviruses (10,11,14,15). However, we did not detect segregation of nucleolar contents into granular and fibrillar regions as reported for other whitefly-transmitted viruses (10,11,15).

All whitefly-transmitted viruses induce similar segregation of nucleolar contents and the formation of fibrillar bodies in the nuclei of infected phloem cells (10,11,15). No such segregation of nucleolar contents was detected in our observations of at least five different batches of MYMD-affected tissues at different stages of infection. However, most nucleoli of infected phloem cells appeared more granulated (Figs. 1, 3b) than those of comparable healthy cells. Although the origin of the fibrillar bodies is unclear, it seems that they are a consequence of the disease. The shape of the fibrillar bodies found in our experiments is very similar to the

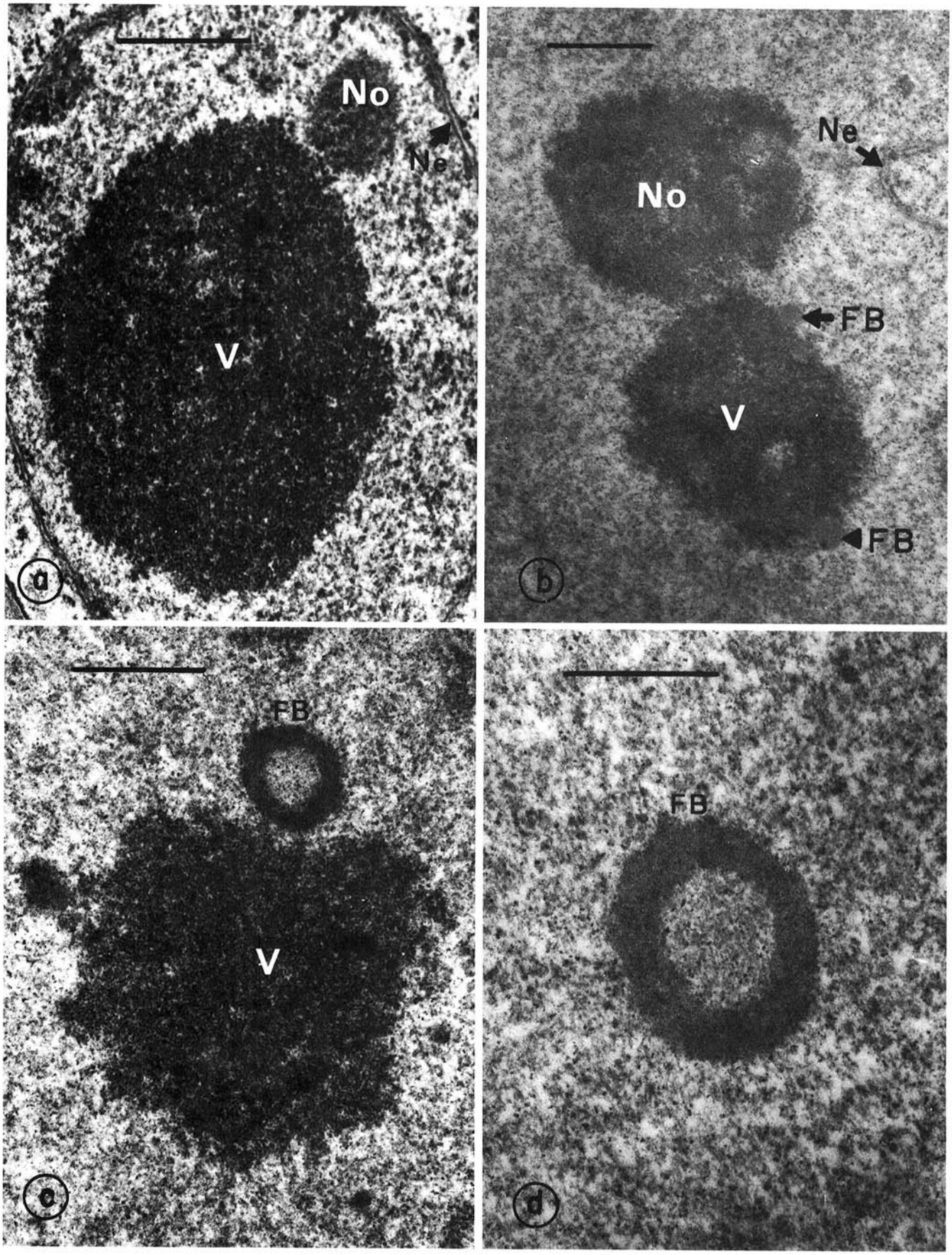

Fig. 3. Inclusions in the nucleus of infected sieve elements of mungbean affected by mungbean yellow mosaic disease; **a,** an aggregate of viruslike particles and small nucleolus; **b,** fibrillar bodies associated with the aggregate of viruslike particles next to the nucleolus; **c,** a fibrillar body with the ringlike appearance associated with an aggregate of viruslike particles; **d,** free fibrillar body in the nucleoplasm. **V,** aggregates of viruslike particles; **No,** nucleolus; **Ne,** nuclear envelope; **FB,** fibrillar bodies. All scale bars = 500 nm.

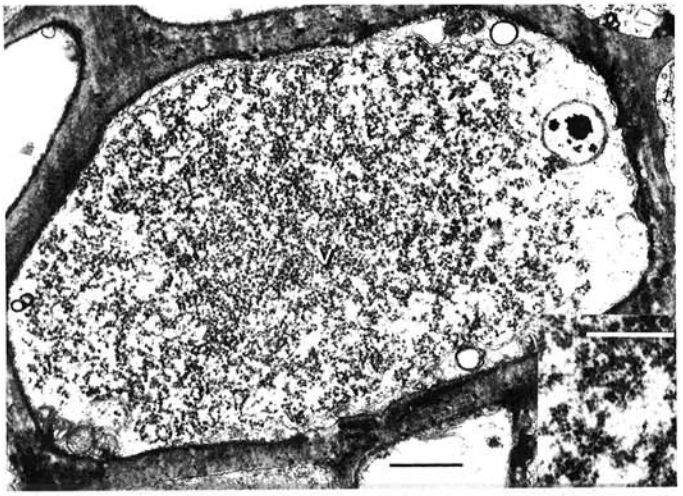

Fig. 4. A fully differentiated sieve element of a mungbean yellow mosaic disease-affected mungbean plant containing scattered viruslike particles (V). Inset shows enlarged viruslike particles. Black scale bar = 1,000 nm, and white scale bar = 200 nm.

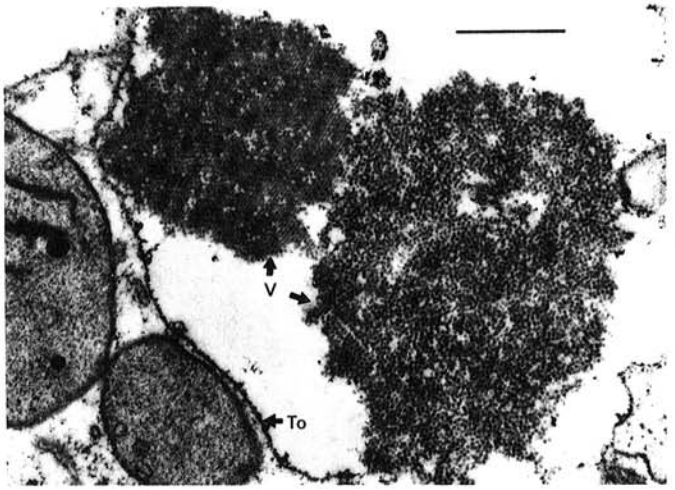

Fig. 5. Paracrystalline and loose aggregates of viruslike particles (V) in the vacuole of a partially differentiated sieve element of a mungbean yellow mosaic disease-affected mungbean plant. To, tonoplast. Scale bar = 500 nm.

fibrillar rings which are found in nuclei of BGMV-infected bean cells (10). Kim et al (10) reported cytochemical evidence indicating that fibrillar rings in BGMV-infected bean cells are deoxyribonucleoprotein which was different from the ribonucleoprotein material of nucleolar contents. These results led them (10) to conclude that the fibrillar rings were not modifications of preexisting nucleoli but were formed after virus infection.

The double cylindrical aggregates of VLPs found in our experiments are somewhat similar to the rod-shaped aggregates of paired particles of BCTV, CSMV, and TLCV (3,4,14). However, the rod-shaped aggregates of paired particles were less organized than the double cylindrical aggregates of MYMD - VLPs and were found only in the nucleoplasm of the infected cells, except for CSMV aggregates which were also in the cytoplasm; other geminiviruses have not been reported in the cytoplasm of infected cells. We observed the double cylindrical aggregates of MYMD-VLPs in tissues sampled as early as the first day and as late as the fifth day after symptom appearance.

Considering the results of the ultrastructural changes induced by the infection of BGMV, EMV, and TLCV together with the findings in our experiments, we agree with Kim et al (10) and Kim and Flores (11) that ultrastructural aspects of host cells may possibly be used as a criterion for diagnosis of diseases caused by whitefly-transmitted geminiviruses.

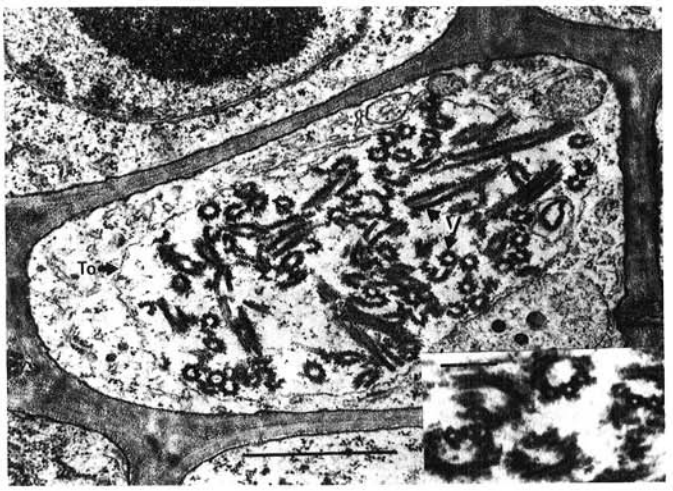

Fig. 6. Double cylindrical aggregates of viruslike particles (V) in the vacuole of an infected sieve element. Inset shows enlarged cross-section view of small cylinders formed by the arrangement of five rows of viruslike particles. The loose aggregate of viruslike particles in the nucleus of a neighboring sieve element is at the top of the figure. To, tonoplast. Large scale bar = 1,000 nm; inset bar = 100 nm.

LITERATURE CITED

1. BIRD, J., and K. MARAMOROSCH. 1978. Viruses and virus diseases associated with whiteflies. Adv. Virus Res. 22:55-110.
2. BOCK, K. R., E. J. GUTHRIE, and R. D. WOODS. 1974. Purification of maize streak virus and its relationship to viruses associated with streak diseases of sugar cane and *Panicum maximum*. Ann. Appl. Biol. 77:289-296.
3. ESAU, K. 1977. Virus-like particles in nuclei of phloem cells in Spinach leaves infected with the curly top virus. J. Ultrastruct. Res. 61:78-88.
4. FRANCKI, R. I. B., T. HATTA, N. E. GRYLLS, and C. J. GRIVELL. 1979. The particle morphology and some other properties of chloris striate mosaic virus. Ann. Appl. Biol. 91:51-59.
5. GOODMAN, R. M. 1977. Single-stranded DNA genome in a whitefly-transmitted plant virus. Virology 83:171-179.
6. GOODMAN, R. M., J. BIRD, and P. THONGMEEARKOM. 1977. An unusual viruslike particle associated with golden yellow mosaic of beans. Phytopathology 67:37-42.
7. HARRISON, B. D., H. BARKER, K. R. BOCK, E. J. GUTHRIE, G. MEREDITH, and M. ATKINSON. 1977. Plant viruses with circular single-stranded DNA. Nature (Lond.) 270:760-762.
8. HATTA, T., and R. I. B. FRANCKI. 1979. The fine structure of chloris striate mosaic virus. Virology 92:428-435.
9. HONDA, Y., and C. MATSUI. 1971. Distribution of tobacco mosaic virus in etiolated tobacco leaf cells infected with two viruses. Phytopathology 61:759-762.
10. KIM, K. S., T. L. SHOCK, and R. M. GOODMAN. 1978. Infection of *Phaseolus vulgaris* by bean golden mosaic virus: Ultrastructural aspects. Virology 89:22-33.
11. KIM, K. S., and E. M. FLORES. 1979. Nuclear changes associated with Euphorbia mosaic virus transmitted by the whitefly. Phytopathology 69:980-984.
12. MUMFORD, D. L. 1974. Purification of curly top virus. Phytopathology 64:136-139.
13. NENE, Y. L. 1972. A survey of viral diseases of pulse crops in Uttar Pradesh. G.B. Pant University Press, Pantnagar, India. 191 pp.
14. OSAKI, T., and T. INOUYE. 1978. Resemblance in morphology and intranuclear appearance of viruses isolated from yellow dwarf diseased tomato and leaf curl diseased tobacco. Ann. Phytopathol. Soc. Jpn. 44:167-178.
15. OSAKI, T., H. KOBATAKE, and T. INOUYE. 1979. Yellow vein mosaic of honeysuckle (*Lonicera japonica* Thunb.), a disease caused by tobacco leaf curl virus in Japan. Ann. Phytopathol. Soc. Jpn. 45:167-178.
16. SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.