Nuclear Changes Associated with Euphorbia Mosaic Virus Transmitted by the Whitefly

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Published with the approval of the director, Arkansas Agricultural Experiment Station, Fayetteville.

This work is part of a collaborative research project between the Department of Plant Pathology, University of Arkansas; the Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, and the Department of Plant Pathology and International Soybean Program, University of Illinois, on whitefly-transmitted plant viruses. The authors are indebted to Dr. R. Gámez, Universidad de Costa Rica, for the identification of the virus in the field.

Accepted for publication 26 March 1979.

ABSTRACT

KIM, K. S., and E. M. FLORES. 1979. Nuclear changes associated with Euphorbia mosaic virus transmitted by the whitefly. Phytopathology 69:980-984.

The nuclear ultrastructure was significantly changed in cells of *Euphorbia heterophylla* naturally infected with the whitefly-transmitted Euphorbia mosaic virus in Costa Rica. Hypertrophy of the nucleolus, segregation of the granular and fibrillar regions of the nucleolus, and appearance of fibrillar rings and virus particles occurred. Infected cells were restricted to the phloem, and virus particles occurred only in the nucleus and lumen of mature sieve elements. These changes were very similar to those observed in cells infected with bean golden mosaic virus, another whitefly-transmitted virus containing a single-stranded DNA genome.

Although whitefly-transmitted plant pathogens have been known for more than 30 yr (12), little progress was made in etiological studies until Gálvez and Castaño (7), Silveira et al (15), Bock and Guthrie (4), Matyis et al (13), Goodman (8,9) and co-workers (10), and Osaki and Inoue (14) reported that spherical, geminate particles, 15–20 nm in diameter, are associated with diseased plants. Goodman (8,9) reported that one of the most widespread whiteflyborne viruses in Central and South America, bean golden mosaic virus (BGMV), contains a single-stranded DNA (ssDNA) genome.

Kim et al (11) found BGMV particles in infected nuclei of bean

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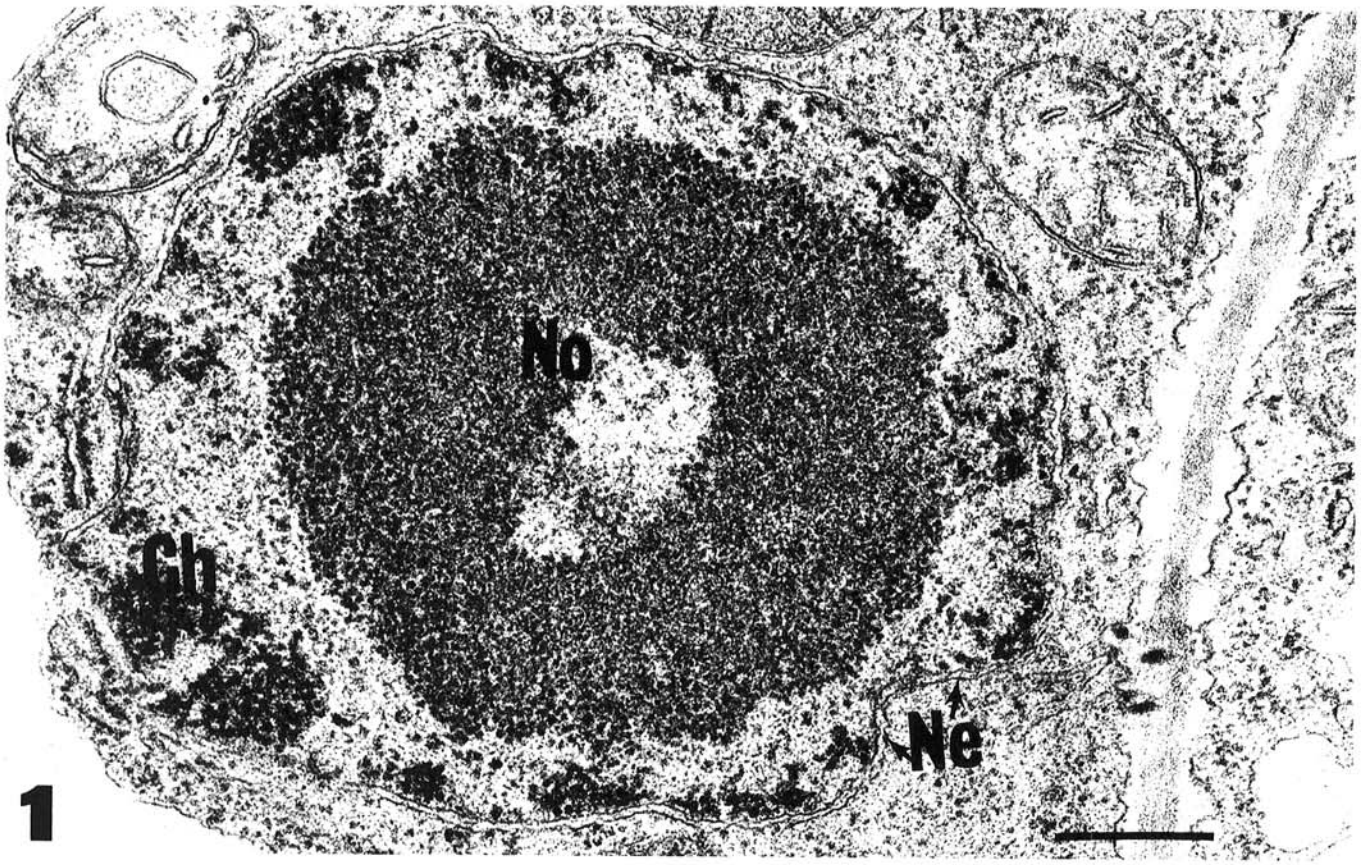

Fig. 1. Nucleus of a phloem parenchymal cell containing hypertrophied nucleolus (No). Ch = chromatin, Ne = nuclear envelope. Scale bar represents 500 nm.

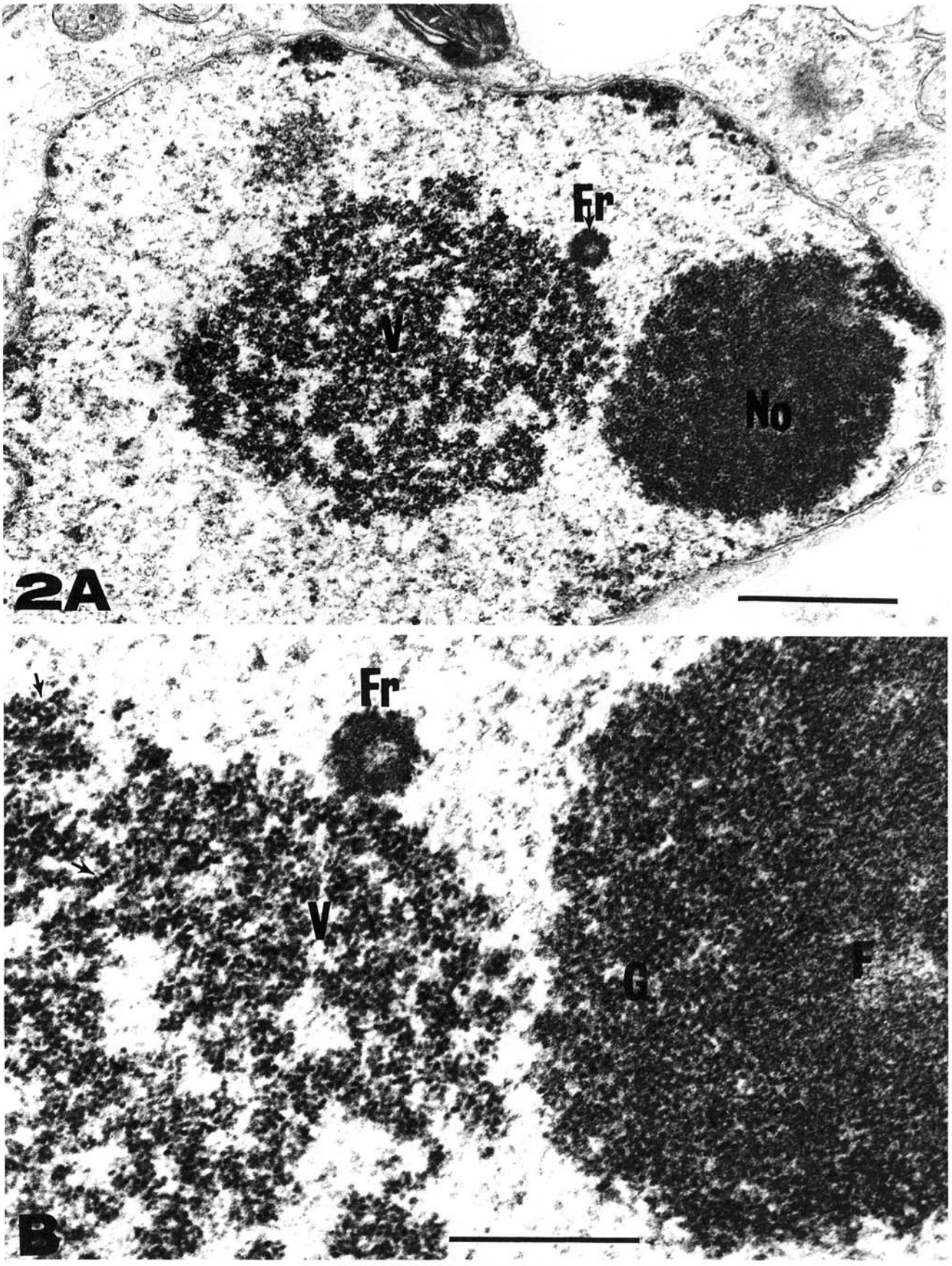

Fig. 2. A, Phloem parenchymal cell nucleus contains large mass of virus particles (**V**), larger in body than its nucleolus (**No**). A small fibrillar ring (**Fr**) is at the outer boundary of the mass of virus particles. Scale bar represents 1,000 nm. **B,** Higher magnification shows the details of the nucleolus, virus particles and fibrillar ring. The granular (**G**) and fibrillar (**F**) portions of the nucleolus are evident. Virus particles are more discrete and smaller than the granules of the nucleolus. Some virus particles are linearly arranged (arrows). Scale bar represents 500 nm.

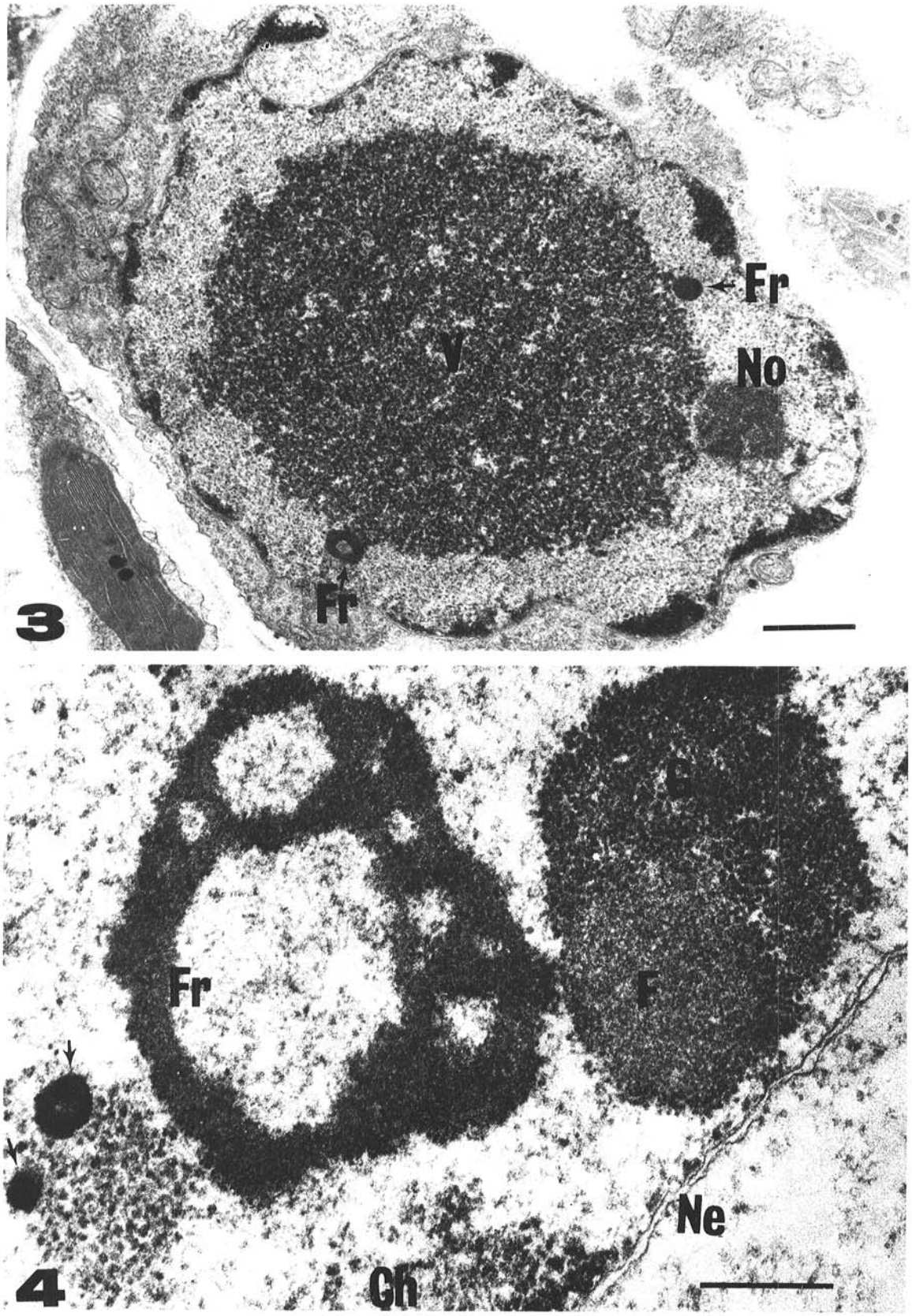

Fig. 3. A nucleus occupied largely by virus particles (**V**). Two small fibrillar rings (**Fr**) are at the outer boundary of the virus mass. The body of the nucleolus (**No**) is extremely small, compared with the virus mass. Scale bar represents 1,000 nm. **Fig. 4.** Portion of nucleus contains the nucleolus and a large (**Fr**) and small (arrows) fibrillar rings. Granular (**G**) and fibrillar (**F**) regions of the nucleolus are segregated. Several electron-lucent holes along the large ring matrix are present. **Ne** = nuclear envelope; **Ch** = chromatin. Scale bar represents 500 nm.

leaf cells and showed that BGMV induces striking changes in nuclear ultrastructure, such as hypertrophy of the nucleolus, segregation of the nucleolar components, appearance of DNA-containing fibrillar rings, and appearance of virus particles. These changes were distinct from those that occur in cells infected with other plant viruses. This, therefore, raised the question of whether other whitefly-transmitted plant pathogens induce nuclear changes similar to those observed in BGMV-infected cells (11).

Euphorbia mosaic virus (3) is another common whitefly-transmitted agent in Central America that is distinctive from BGMA by symptomatology and host range (2,5,6). This paper describes the nuclear ultrastructure of leaf cells of *Euphorbia heterophylla* L. infected with Euphorbia mosaic virus and illustrates the similarity to BGMV-infected bean leaf cells (11).

MATERIALS AND METHODS

E. heterophylla plants with symptoms typical of Euphorbia mosaic were collected at the Centro Agronómico Tropical de Investigación y Enseñanza at Turrialba, Costa Rica. Leaves of various ages of plants with symptoms were fixed in 4% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.0, at the sites of collection and brought to the University of Arkansas, Fayetteville. Unfixed specimens also were brought to the Centro de Investigación en Biología Celular y Molecular at the University of Costa Rica where it was verified that the virus was whitefly-transmitted and where additional specimens of both uninfected and infected leaves were fixed. Specimens were processed for observation with the electron microscope at both universities as described previously (11).

RESULTS AND DISCUSSION

The changes in infected leaves occurred in the nuclei of young and mature sieve elements and associated parenchymal cells. The changes included hypertrophy of the nucleolus (Fig. 1), segregation of granular and fibrillar components of the nucleoli (Fig. 4), and appearance of fibrillar rings (Figs. 2, 3, 4, and 5) and viruslike particles (Figs. 2, 3, and 5). These features were not observed in comparable cells of healthy plants. No nuclear changes of viruslike particles were observed in epidermal, mesophyll, or xylem cells of infected leaves.

Particles, 18–20 nm in diameter, which were assumed to be the virus, occurred as large, compact, and somewhat circular masses only in nuclei. The particles often occupied up to three-fourths of the nuclear volume (Fig. 3). When the nuclei contained such a large amount of virus particles, the nucleoli usually were smaller than the masses of virus particles (Figs. 2 and 3). This suggest that the hypertrophied nucleoli seen in early stages of infection apparently decrease in size as infection progresses. This phenomenon also was observed in the nuclei of BGMV-infected cells (11). A linear arrangement of particles in the large masses was often evident (Fig. 2B).

Fibrillar rings, which varied in size and number, were composed of electron-dense and extremely compact, fine fibrils (Fig. 5B). They usually were closely associated with the outer boundary of the masses of virus particles (Figs. 2 and 3). When several fibrillar rings occurred in a nucleus, some were near the masses of virus particles and others were far from them (Fig. 5A). Fibrillar rings also were observed in nuclei with no virus particles (Fig. 4). The matrices of smaller fibrillar rings were homogeneously electron-dense (Figs. 2,3, and 5), whereas those of larger ones contained electron-lucent holes of various sizes along the ring matrices (Fig. 4).

The types of cells involved in infection, the site, form, and sizes of virus particles, presence of nucleolar segregation, and formation of fibrillar rings are almost indistinguishable from those previously observed in cells infected with BGMV (11). Similar nuclear changes also were found in cells of human kidney culture and of hamster embryo infected with ssDNA animal virus (1,16).

Silveira et al (15) observed geminate particles in dip preparations made from Puerto Rican Euphorbia mosaic virus-infected plants, which were similar in morphology and size to those from BGMV-infected hosts (7,9). Because these two whitefly-transmitted viruses also induce similar ultrastructural changes in situ in different hosts, we suggest that Euphorbia mosaic virus, and perhaps the other

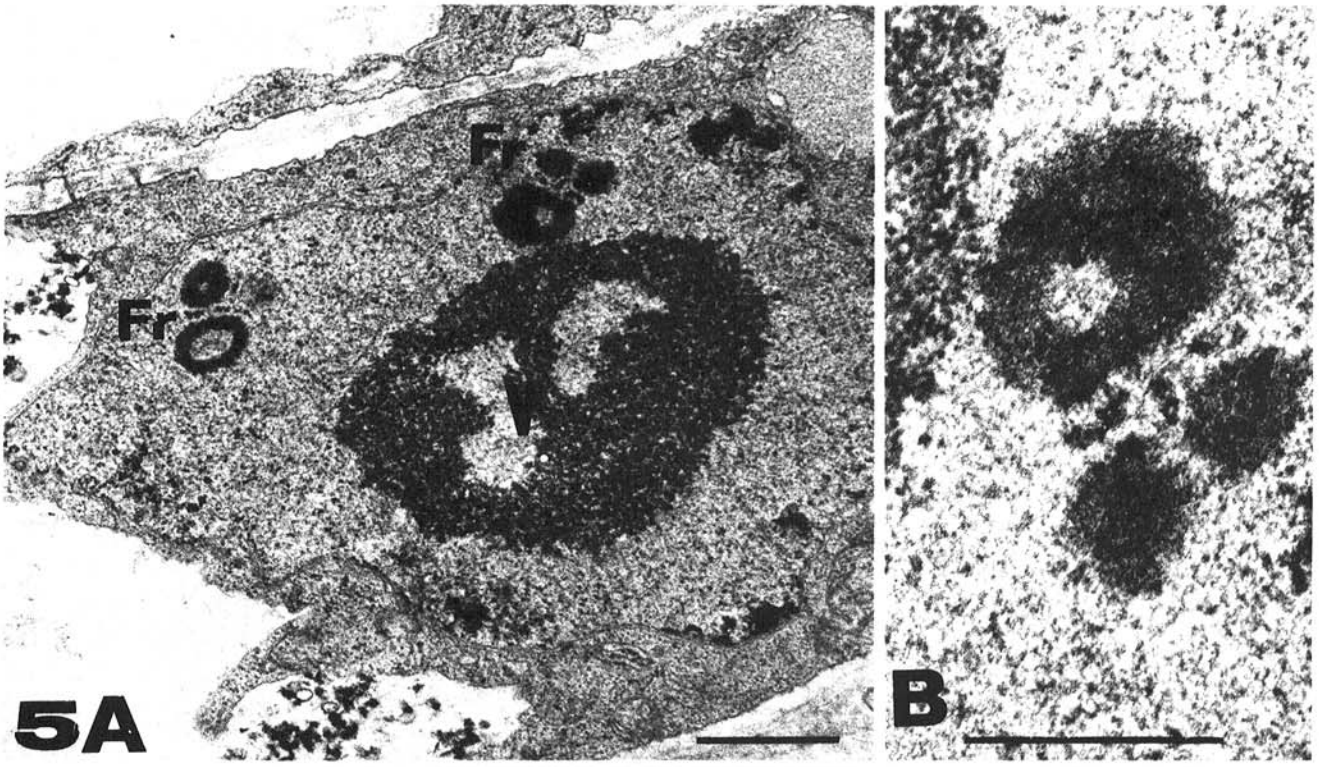

Fig. 5. A, Nucleus contains a large mass of virus particles (**V**) and five fibrillar rings (**Fr**). Three fibrillar rings are in the vicinity of the mass of virus particles; the other two are apart from it. Scale bar represents 1,000 nm. **B**, Higher magnification shows detail of the fibrillar rings. Scale bar represents 500 nm.

whitefly-transmitted viruses, contain ssDNA. If this is true, the structural changes in nuclei induced by these two viruses may be characteristic of ssDNA viruses.

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