Cytopathology and Transmission Characteristics of a Virus Isolate from *Abutilon striatum*

V. B. V. de Souza and K. S. Kim

Department of Plant Pathology, University of Arkansas, Fayetteville 72701. Current address of first author: Centro de Microscopia Eletronica, Universidade Federal do Parana, 800.000-Curitiba, PR, Brazil. Reprint requests should be addressed to the second author.

Portion of a dissertation submitted by the first author for a Ph.D. degree.
Supported in part by Brazilian National Research Council (CNPq-200.411/85) and Universidade Federal do Parana, Brazil.
Published with the approval of the Director of the Arkansas Agricultural Experiment Station, Fayetteville.
Accepted for publication 18 December 1989 (submitted for electronic processing).

ABSTRACT

de Souza, V. B. V., and Kim, K. S. 1990. Cytopathology and transmission characteristics of a virus isolate from *Abutilon striatum*. Phytopathology 80:548-552.

Comparative studies were made of the symptomatology, transmission, and ultrastructure of a United States virus isolate (U.S. isolate) from *Abutilon striatum* that had been propagated vegetatively for many years and a Brazilian isolate of Abutilon mosaic virus from naturally infected *Malva parviflora* and *Sida micrantha*. The U.S. isolate differed from the Brazilian isolate in that it never induced in the experimental hosts the characteristic angular mosaic described for the Brazilian isolate, was not mechanically or vector transmitted, and induced striking and unique cytopathological changes in the infected cells of the original host as well as in graft-infected *A. pictum*, *M. parviflora*, and *S. rhombifolia*.

Additional keywords: Abutilon variegation mosaic, geminivirus, ultrastructure.

Abutilon mosaic was first identified as a disease in 1868 after a variegated plant of *Abutilon striatum* Dicks. was imported from the West Indies to Europe and vegetatively propagated for commercial purposes (10,14). Because the disease was not naturally disseminated in Europe, the variegation was thought to be induced genetically until its infectious character was demonstrated through grafting by several investigators (10,14,21). Although Abutilon mosaic was one of the earliest plant diseases now known to be caused by a virus, very little was known about its causal agent for more than a century (3,4).

Transmission of Abutilon mosaic by the whitefly *Bemisia tabaci* Genn. was demonstrated for the first time by Orlando and Silberschmidt (26), and mechanical transmission was successfully demonstrated 14 yr later by Costa and Carvalho (9). The results of transmission experiments supported the idea that the disease agent was a virus, but viruslike particles were not seen until 1974 when Costa observed them in the phloem of diseased *Sida* spp. plants (7). Costa (8) suggested that the disease agent should be included with viruses in what was later called the geminivirus group. Recent studies on ultrastructural and biochemical aspects of Abutilon mosaic virus (AbMV) support this classification (1,2,17–20).

During the past 60 yr, several differences have been reported in host range, symptomatology, and transmission characteristics of AbMV isolates from different parts of the world. These observations suggested that indeed there was not one but several viruses causing the disease complex which has been referred to as infectious chlorosis of Malvaceae (4,6,8,9,14,16,21,28–30). Flores and Silberschmidt (14) presented evidence that the causal agents of infectious chlorosis of Malvaceae and Abutilon mosaic were not the same and that the causal agent of the mosaic of *A. striatum* from the United States, which had been propagated by cuttings for more than a century, differed from the agent causing mosaic in *A. striatum* in Brazil.

This paper presents the results of investigations on AbMV collected in the United States and Brazil with emphasis on the ultrastructural aspects. Evidence is presented that the U.S. isolate is quite distinct from the naturally occurring Brazilan isolate. Preliminary results of this study have been reported (11).

MATERIALS AND METHODS

Viruses. Variegated plants of *A. striatum* that had been propagated vegetatively in nurseries for ornamental use in the United States (U.S. isolate) for many years were used in this study. For comparison, samples were collected in Brazil (Brazilian

isolate) from naturally infected plants of *Malva parviflora* L. and *Sida micrantha* St. Hil.

Transmission tests. Graft transmission tests were done using material infected by the U.S. isolate. Infected leaves and leaf buds of *A. striatum* were grafted onto young, healthy plants of *A. pictum* Walp, *Gossypium hirsutum* L., *Hibiscus esculentus* L., *M. parviflora* L., *S. carpinifolia* L., *S. cordifolia* L., *S. rhombifolia* L., and *S. santaremnensis* H. Mont. *Abutilon* plants were propagated by cuttings (the *Abutilon* species were identified by E. B. Smith, Department of Botany and Microbiology, University of Arkansas, Fayetteville). Seeds of *H. esculentus* were donated by Honjo Cia. Ltda., Curitiba, PR, Brazil; those of the other Malvaceae were supplied by Vismar da Costa Lima Neto, Universidade Federal do Parana, Brazil. A total of 20 plants of each species was grafted on two different occasions.

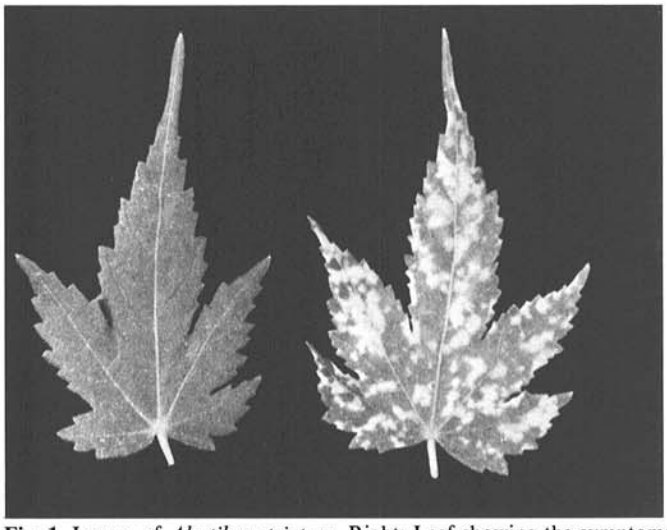

Fig. 1. Leaves of *Abutilon striatum*. Right: Leaf showing the symptoms of infection by the U.S. isolate of Abutilon mosaic virus. Left: uninfected leaf.

For the mechanical inoculation tests, leaf tissue from AbMV-infected plants was ground in a mortar with a pestle at room temperature in proportions of 1:5 (w/v) with 0.02 M phosphate buffer, pH 7.0, and with or without 0.02 M sodium sulfite, as described by Costa and Carvalho (9) and Flores and Silberschmidt (14). Leaves of plants of *A. striatum* infected by the U.S. isolate were used initially, but because *Abutilon* spp. sap was known to contain inhibitors of virus transmission (25), leaves from *M. parviflora* and *S. rhombifolia* that had been infected by grafting with the U.S. material were used later. The inoculum was rubbed onto Carborundum-dusted seedlings of the species listed in the previous paragraph. In five separate experiments, a total of 50 plants of each species was inoculated.

For vector transmission tests, the whitefly *B. tabaci*, the vector of AbMV in areas of natural dissemination of the disease, was used. On four different occasions, 10 groups of 60 caged insects were allowed acquisition access periods of 24–72 hr on naturally infected *A. striatum* and on graft-infected plants of *M. parviflora* and *S. rhombifolia*. The whiteflies were transferred to healthy,

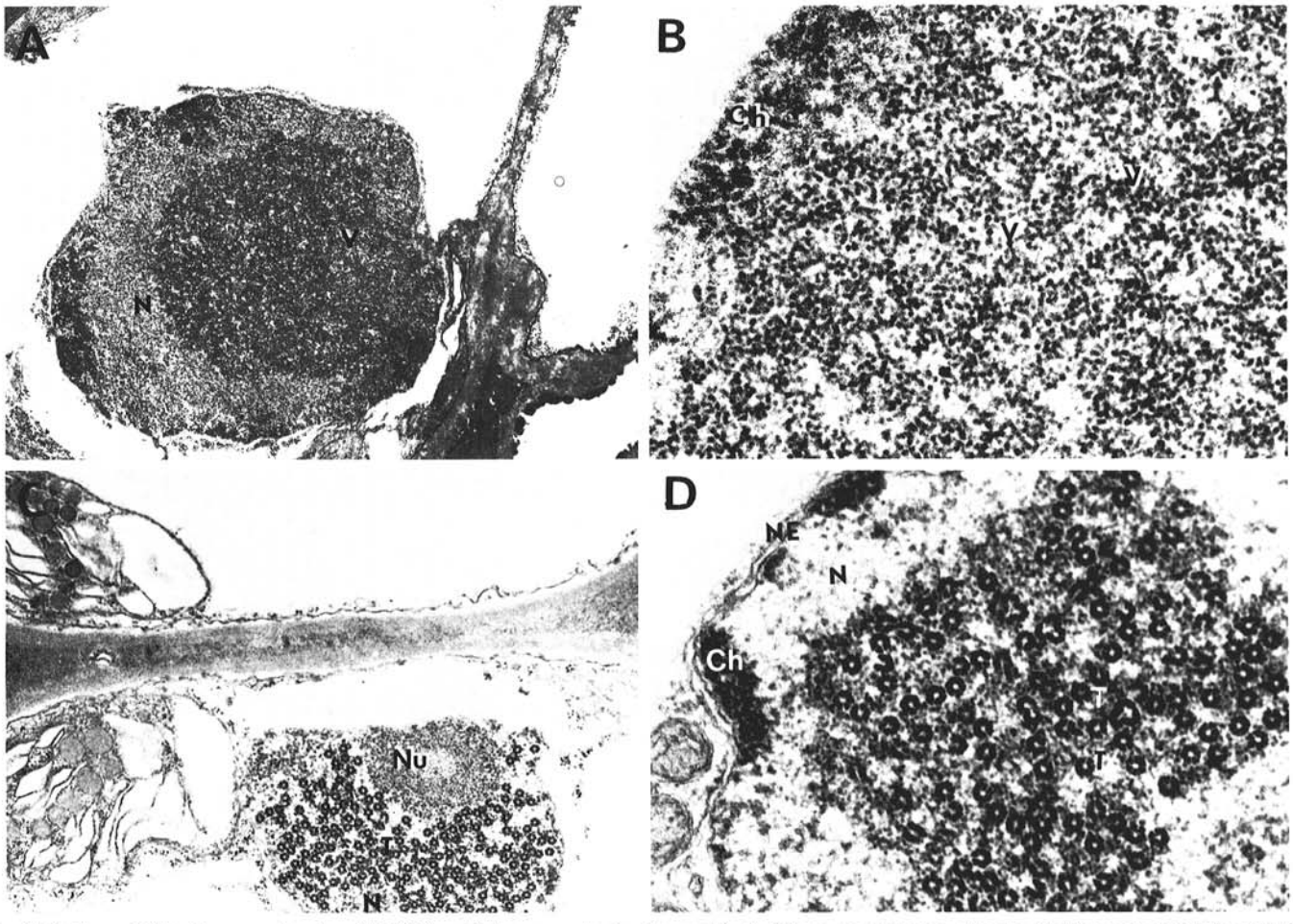

Fig. 2. Pattern of Abutilon mosaic virus (AbMV) particle aggregates in the nuclei of phloem parenchyma cells of *Abutilon striatum* infected with U.S. isolate of AbMV. **A,** Low magnification of a nucleus (N) containing randomly arranged aggregates of AbMV particles (V). ×10,000. **B,** Higher magnification of a nucleus exhibiting details of randomly arranged particles of AbMV (V). Ch = chromatin. ×60,000. **C,** Low magnification of a nucleus (N) containing AbMV particles forming tubular structures (T). Nu = nucleolus. ×17,000. **D,** Transverse section of the tubular structures (T) which appeared as rings with each ring having an external wall encircling an electron-lucent lumen. N = nucleus; NE = nuclear envelope; Ch = chromatin. ×39,000.

young plants of *A. pictum*, *M. parviflora*, and *S. rhombifolia* for inoculation access periods of 3–7 days. Longer periods were not tried because transmission rates are known to decrease with time (8). A total of 2,400 insects and 40 plants was used. In all transmission tests, experimental plants were kept in the greenhouse and inspected visually for development of symptoms for up to 6 mo.

Electron microscopy. Leaf tissues from infected plants were processed for transmission electron microscopy as described by Kim and Fulton (23). The samples were collected from the two groups of plants mentioned: the vegetatively propagated *A. striatum* infected by the U.S. isolate plus the material infected by grafting with this plant (*M. parviflora*, *A. pictum*, and *S. rhombifolia*), and *M. parviflora* and *S. micrantha* naturally infected by the Brazilian isolate. The latter samples were processed for electron microscopy in the Electron Microscopy Laboratory of the University of Brasilia, Brazil, and brought to the Department of Plant Pathology of the University of Arkansas, Fayetteville, for the ultrastructural studies.

Thin sections were double stained with 2% aqueous uranyl acetate and lead citrate for 10–15 min in each stain. Observations were made in a JEOL 100 CX (JEOL [U.S.A.] INC., Peabody, MA) electron microscope.

RESULTS

Transmission. The U.S. isolate of AbMV was transmitted only by grafting to *A. pictum*, *M. parviflora*, and *S. rhombifolia*. Despite the use of heterografts, most grafts took well; however, less than 8% (12 out of a total of 160) of the plants tested were infected. *M. parviflora* was the most susceptible species. Symptom expression was delayed 2 mo in *M. parviflora* and up to 5 mo in *A. pictum* and *S. rhombifolia*. In all plants infected, only one or a few branches showed symptoms, and, in most cases, the first branches to show symptoms were situated below the graft point. The virus was not transmitted mechanically or by *B. tabaci* to any of the test plants.

Symptoms. The infected plants of *A. striatum* from the United States exhibited an intense mosaic composed of large areas of golden, yellow, or light green scattered throughout the leaf. These areas had no defined shape and were not confined to any special region of the leaves (Fig. 1). These same symptoms were reproduced in the graft-infected plants, but they were less severe in *A. pictum* than in the other hosts. *M. parviflora* showed the most severe symptoms which included leaf crinkling and distortion. The plants collected in Brazil displayed the angular mosaic symptoms of the infectious chlorosis of Malvaceae described by Silberschmidt (28), Costa and Carvalho (9), and Flores and Silberschmidt (14).

Electron microscopy. In thin sections of leaves of *A. striatum* infected by the U.S. isolate, viruslike particles of an average diameter of 16 nm usually in large aggregates were observed only in the nuclei of phloem parenchyma cells. These aggregates either were packed randomly (Fig. 2A and B) or often were arranged orderly into tubular structures measuring approximately 80 nm in diameter (Figs. 2C and D and 3A–C). In transverse sections, these tubules appeared as electron-dense rings surrounding an electron-lucent lumen (Fig. 2D). The ring was composed of particles symmetrically arranged in six triangles with the apices to the inside. In this way, the particles appeared arranged in two rows around the circumference of the tubules with 12 particles in the external and six in the internal row (Fig. 3A, diagram, and B). In some instances, the tubules were merged in groups of two, three, or four, forming more complex structures (Fig. 3B). In longitudinal views of the tubules, which were of variable lengths, the virus particles appeared to be aligned in a zigzag pattern along the tubule walls (Fig. 3C). The same tubular structures found in the samples of *A. striatum* were observed in the graft-infected material of *A. pictum*, *M. parviflora*, and *S. rhombifolia*.

In the naturally infected material from Brazil, however, the complex crystalline tubular structures of virus particles observed in *A. striatum* infected with the U.S. isolate were not present. Thin sections of these samples revealed the presence of high numbers of viruslike particles in nuclei of infected cells in the vascular region; however, these were mostly packed without orderly arrangement. In a few cases, particles were seen aligned in single, double, or triple rows (Fig. 3D, arrows). Circular profiles that could suggest cross-sectional tubules were never observed.

None of the structures described above was found in healthy samples of *A. pictum*, *M. parviflora*, or *S. rhombifolia*.

DISCUSSION

Results of the transmission experiments indicated that the U.S. isolate of AbMV behaved differently from the Brazilian isolates studied by Costa (6), Costa and Carvalho (9), and Flores and Silberschmidt (14). Symptomatology and ultrastructural changes of infected tissues induced by the U.S. and Brazilian isolates of AbMV also differed.

M. parviflora has been reported to be an excellent source and host species for mechanical transmission tests of the Brazilian isolates of AbMV (9); however, all attempts to mechanically transmit the U.S. AbMV isolate failed, not only when the original *A. striatum* was used as the source of inoculum, but also when the graft-infected *M. parviflora* and *S. rhombifolia* were tested. This is consistent with the suggestion by Costa and Carvalho (9) that different strains of AbMV differ in their ability to be transmitted mechanically.

Similarly, *S. rhombifolia* was reported to be a very susceptible host to insect transmission of the Brazilian AbMV (13,26); however, no positive results were obtained from the tests with the U.S. isolate, even when the same source and test plants were used. Under the conditions reported here, the U.S. virus isolate was not transmitted mechanically or by whitefly; it was transmitted only with relative difficulty by grafting. The behavior of this U.S. isolate differs also from the U.S. isolate studied by Flores and Silberschmidt (14) which was transmitted mechanically and by whitefly .

Difficulties in obtaining vector transmission of a U.S. isolate of AbMV led Black (5) to suggest that the long-term vegetative propagation of AbMV-infected *A. striatum* in the United States may have led to the loss of transmissibility by the vector. In this regard, this study suggests that the long vegetative propagation of AbMV-infected plants of *A. striatum* also may have had an effect on the mechanical transmissibility of the virus and on its relationship with the hosts perhaps by selecting populations of the virus that are only graft or vegetatively propagated within the host.

Brazilian AbMV causes an angular mosaic in leaf areas between the secondary veins of *A. striatum*, *M. parviflora*, and *Sida* spp. (9,14,28). Angular mosaic also was described in the *Sida* spp. found in Puerto Rico infected with the infectious chlorosis of Malvaceae by means of the natural vector (3). This mosaic is different from that observed in the material infected with the U.S. virus isolate which never displayed an angular mosaic pattern.

Particle size and morphology of AbMV and the higher concentration of particles in phloem tissues are characteristic of the geminiviruses. However, fibrillar bodies known to be induced by many whitefly-transmitted geminiviruses (22–24) were not encountered in any of the hosts. The present observations on the ultrastructure of AbMV-infected cells clearly allow separation of the samples into two cytopathological groups which correspond to the two transmission and symptomatology groups: the group made up of the field-infected material brought from Brazil, and the group consisting of U.S. AbMV-infected *A. striatum* and the three other malvaceous species infected by grafting from this plant. The latter group presented very complex virus particle arrangements in tubular structures, whereas the former group showed a simple arrangement of a few rows of particles.

Jeske et al (17), Jeske and Schuchalter-Eicke (18), Abouzid and Jeske (2), and Abouzid et al (1) described the ultrastructure of AbMV in the following material: *Abutilon sellovianum* Reg. from Germany, *S. micrantha* from Brazil infected via vector or

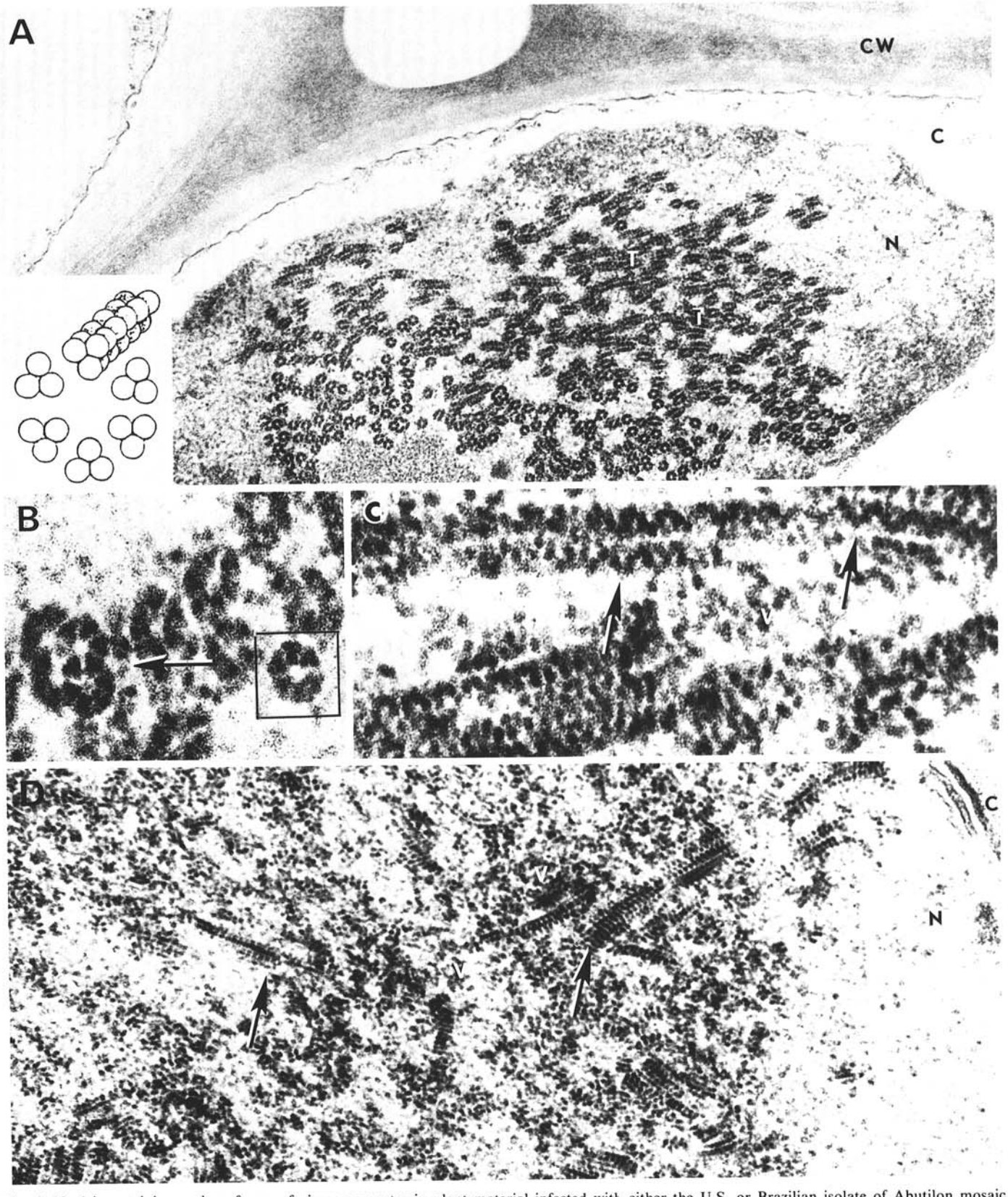

Fig. 3. Nuclei containing various forms of virus aggregates in plant material infected with either the U.S. or Brazilian isolate of Abutilon mosaic virus (AbMV). **A,** A nucleus (N) of *Abutilon striatum* infected with the U.S. isolate of AbMV containing virus tubules (T) sectioned both transversely and obliquely. C = cytoplasm; CW = cell wall. ×26,000. Lower left is a proposed model for the arrangement of the virus particles as viewed in cross section with a perspective of the walls. Refer also to a tubule in the square in Figure 3B. **B,** Higher magnification of the tubules in Figure 3A showing the details of particle arrangement. The circumference of each tubule is made of 18 particles arranged in six triplets (see square and also the diagram in Fig. 3A) so that the outer wall is composed of 12 particles and the inner wall (surrounding the lumen) is composed of six particles. Some tubules are merged, forming larger and more complex tubules (arrow). ×150,000. **C,** A nucleus from *Malva parviflora* infected by grafting with the U.S. isolate of AbMV showing a higher magnification of the virus tubules sectioned longitudinally. The zigzag arrangement of the particles along the tubule walls is evident (arrows). V = virus particles. ×150,000. **D,** A nucleus (N) from *Sida micrantha* field infected with the Brazilian isolate of AbMV. Compare the crystalline arrangement in rows (arrows) with the tubular arrangement shown in the previous figures. C = cytoplasm; V = virus particles. ×60,000.

mechanically, and *M. parviflora* and *M. sylvestris* L. mechanically inoculated with inoculum from *S. micrantha*. An analysis of their results suggests conclusions similar to ours. In the samples from Brazil, Jeske et al (17) described cytopathic effects comparable to those described in the Brazilian material studied in this paper. In *A. sellovianum* (that had not been transmitted mechanically), however, Jeske and Schuchalter-Eicke (18) described viruslike particles arranged in tubules which are similar, although not identical, to the ones encountered in the *A. striatum* from the United States and in the hosts that were graft infected by this isolate. In *A. sellovianum*, 18 particles also were found in the circumference of the tubules, but these particles were arranged in single rows; the diameter of the tubules is expectedly larger (80–120 nm) than in the material infected with the U.S. isolate. These differences, if not due to host effect, could indicate the presence of different isolates of AbMV in these two species of *Abutilon*.

A crystalline arrangement of virus particles has been found in plants infected with other geminiviruses: the *Digitaria* strain of maize streak virus (12), tomato golden mosaic virus (27), bean golden mosaic virus (24), Chloris striate mosaic virus (15), and Euphorbia mosaic virus (22), but none forms tubules like those detected in U.S. AbMV-infected plants of *Abutilon*.

The observation that the virus particles form large, complex aggregates may provide an explanation for the lack of mechanical and vector transmission because this arrangement of particles may cause a decrease in the effective concentration of particles available for acquisition/inoculation, which is already very low (1.5–14 μg of virus/g of tissue as determined by Abouzid and Jeske [2]).

The reported differences in transmissibility, symptoms, and cytopathology of AbMV isolates from different sources add data for the characterization of the members of the group of causal agents of the infectious chlorosis of Malvaceae. More studies on this material could reveal the existence of mixtures of strains in some of the vegetatively propagated material studied, as suggested by Costa (8) and Flores and Silberschmidt (14).

The extent to which the U.S. isolate of AbMV associated with long-term vegetative propagation of the host plant differs from the native Brazilian isolate is not known; however, the lack of transmissibility by means other than grafting, the absence of fibrillar bodies, and the unique cytopathology associated with the infection of the host plants by the U.S. isolate are not characteristic of the whitefly-transmitted geminiviruses.

LITERATURE CITED

1. Abouzid, A. M., Barth, A., and Jeske, H. 1988. Immunogold labelling of the Abutilon mosaic virus in ultrathin sections of epoxy resin embedded leaf tissue. J. Ultrastruct. Mol. Struct. Res. 99:38-47.
2. Abouzid, A., and Jeske, H. 1986. The purification and characterization of gemini particles from Abutilon mosaic virus infected malvaceae. Phytopathol. Z. 115:344-353.
3. Bird, J. 1958. Infectious chlorosis of *Sida carpinifolia* in Puerto Rico. Tech. Pap. Agric. Exp. Stn., Rio Piedras, P. R.
4. Bird, J., and Maramorosch, K. 1978. Viruses and virus diseases associated with whiteflies. Adv. Virus Res. 22:55-110.
5. Black, L. M. 1953. Loss of vector transmissibility by viruses normally insect transmitted. (Abstr.) Phytopathology 43:466.
6. Costa, A. S. 1955. Studies on Abutilon mosaic in Brazil. Phytopathol. Z. 24:97-112.
7. Costa, A. S. 1975. Increase in the populational density of *Bemisia tabaci*, a threat of widespread virus infection of legume crops in Brazil. Pages 27-49 in: Tropical Diseases of Legumes. Academic Press, New York.
8. Costa, A. S. 1976. Whitefly-transmitted plant diseases. Annu. Rev. Phytopathol. 16:429-449.
9. Costa, A. S., and Carvalho, A. M. 1960. Mechanical transmission and properties of the *Abutilon* mosaic virus. Phytopathol. Z. 37:259-272.
10. Davis, E. F. 1929. Some chemical and physiological studies on the nature and transmission of "infectious chlorosis" in variegated plants. Ann. Mo. Bot. Gard. 16:145-227.
11. de Souza, V. B. V., and Kim, K. S. 1988. Ultrastructural aspects of abutilon mosaic virus. (Abstr.) Phytopathology 78:1548.
12. Dollet, M., Accotto, G. P., Lisa, V., Menissier, J., and Boccardo, G. 1986. A geminivirus, serologically related to maize streak virus, from *Digitaria sanguinalis* from Vanuatu. J. Gen. Virol. 67:933-937.
13. Flores, E., and Silberschmidt, K. 1958. Relations between insect and host plant in transmission experiments with infectious chlorosis of malvaceae. An. Acad. Bras. Cienc. 30:535-560.
14. Flores, E., and Silberschmidt, K. 1967. Contribution to the problem of insect and mechanical transmission of infectious chlorosis of Malvaceae and the disease displayed by *Abutilon thompsonii*. Phytopathol. Z. 60:181-195.
15. Francki, R. I. B., Hatta, T., Gryllis, N. E., and Grivell, C. J. 1979. The particle morphology and some other properties of chloris striate mosaic virus. Ann. Appl. Biol. 91:51-59.
16. Hertzsch, W. 1927. Beitrage zur Infektiosen Chlorose. Z. Bot. 20:65-85.
17. Jeske, H., Menzel, D., and Werz, G. 1977. Electron microscopic studies on intranuclear virus-like inclusions in mosaic-diseased *Abutilon sellowianum* Reg. Phytopathol. Z. 89:289-295.
18. Jeske, H., and Schuchalter-Eicke, G. 1984. The *Abutilon* mosaic virus (AbMV) in different leaf tissues of several host species of Malvaceae. Phytopathol. Z. 109:353-362.
19. Jeske, H., and Werz, G. 1978. The influence of light intensity on pigment composition and ultrastructure of plastids in leaves of diseased *Abutilon sellowianum* Reg. (Malvaceae). Phytopathol. Z. 91:1-13.
20. Jeske, H., and Werz, G. 1980. Ultrastructural and biochemical investigations on the whitefly transmitted *Abutilon* mosaic virus (AbMV). Phytopathol. Z. 97:43-55.
21. Keur, J. Y. 1934. Studies on the occurrence and transmission of virus diseases in the genus Abutilon. Bull. Torrey Bot. Club 61:53-70.
22. Kim, K. S., and Flores, E. M. 1979. Nuclear changes associated with Euphorbia mosaic virus transmitted by the whitefly. Phytopathology 69:980-984.
23. Kim, K. S., and Fulton, R. W. 1984. Ultrastructure of *Datura stramonium* infected with an Euphorbia virus suggestive of a whitefly-transmitted geminivirus. Phytopathology 74:236-241.
24. Kim, K. S., Shock, T. L., and Goodman, R. M. 1978. Infection of *Phaseolus vulgaris* by bean golden mosaic virus: Ultrastructural aspects. Virology 89:22-33.
25. Moraes, W. B. C., Martins, E. M. F., Conti, E., and Oliveira, A. R. 1974. The inhibitory activity of extract of *Abutilon striatum* leaves on plant virus infection. Phytopathol. Z. 81:145-152.
26. Orlando, A., and Silberschmidt, K. 1946. Estudos sobre a disseminacao natural do virus da "clorose infecciosa" das malvaceas (abutilon virus I. Baur) e sua relacao com o inseto-vetor "*Bemisia tabaci* (Genn.)." (Homoptera-Aleyrodidae). Arq. Inst. Biol. Sao Paulo 17:1-35.
27. Rushing, A. E., Sunter, G., Gardiner, W. E., Dute, R. R., and Bisaro, D. M. 1987. Ultrastructural aspects of tomato golden mosaic virus infection in tobacco. Phytopathology 77:1231-1236.
28. Silberschmidt, K. 1943. Estudos sobre a transmissao experimental da "clorose infecciosa" das malvaceas. Arq. Inst. Biol. Sao Paulo 14:105-157.
29. Silberschmidt, K., Flores, E., and Tommasi, L. R. 1957. Further studies on the experimental transmission of "Infectious Chlorosis of Malvaceae." Phytopathol. Z. 30:387-414.
30. Silberschmidt, K., and Tommasi, L. R. 1955. Observacoes e estudos sobre especies de plantas susceptiveis a clorose infecciosa das malvaceas. An. Acad. Bras. Cienc. 27:195-214.