A Unique Virus Isolated from Elephant Grass

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ABSTRACT

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A virus was isolated from leaves of elephant grass plants (*Pennisetum purpureum*) showing mosaic symptoms. It was mechanically transmitted to a few cultivars of corn (*Zea mays*) and sorghum (*Sorghum bicolor*), but other test plants, including elephant grass, could not be infected. The virus was not transmitted by *Myzus persicae* or *Rhopalosiphum maidis*. Antiserum with a titer of 1:4,096 in a microprecipitin test was obtained with virus purified from elephant grass. Antisera against several potyviruses did not react with this virus in double-diffusion or serologically specific electron microscopy. The purified virus had a single capsid protein of 36 kDa. Although viral nucleic acid could not be obtained for characterization, dsRNA of 6.7 mDa was isolated from infected tissues. Elongated virus particles were localized in the cytoplasm by immunocytochemistry. Electron dense tubular material of an unknown nature, in addition to the lamellar inclusions of potyviruses of subgroup I, was also present in the cytoplasm of infected cells. This virus seems to be distinct from previously described potyviruses.

Elephant grass (*Pennisetum purpureum* Schumach.) is a perennial used as forage for cattle throughout the world (13) and is widely distributed in Brazil. Only the maize streak geminivirus has been reported to naturally infect elephant grass (2); experimentally, however, elephant grass is susceptible to sugarcane mosaic virus (6). During a survey of corn virus diseases in the state of Parana, elephant grass showing conspicuous mosaic symptoms (Fig. 1A) was found. This paper reports the viral nature of this elephant grass disease and partial characterization of the causal agent.

MATERIALS AND METHODS

Virus. Cuttings collected from naturally infected elephant grass in Santa Helena County, in the state of Parana, were established under greenhouse conditions at the University of Brasilia. The source of virus for all studies was from these clones.

Host range. Test plants used in host range studies included *Gomphrena globosa* L. (Amaranthaceae); *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn. (Chenopodiaceae); *Andropogon schoenanthus* L., *Avena sativa* L., *Hordeum vulgare* L., *Oryza sativa* L., *Panicum compressum* Forsk., *P. maximum* Jacq., *Pennisetum purpureum*, *Secale cereale* L., *Stenotaphrum secundatum* (Walter) Kuntze, *Sorghum bicolor* (L.) Moench, *Triticum aestivum* L., and *Zea mays* L. (Gramineae); *Mucuna sloanei* Fawcett & Rendle, *Phaseolus vulgaris* L., and *Vigna unguiculata* (L.) Walp. (Leguminosae); and *Datura stramonium* L., *Nicandra physalodes* (L.) Gaertn., *Nicotiana tabacum* L., *N. glutinosa* L., and *Physalis floridana* Rydb. (Solanaceae). Inoculum consisted of elephant grass sap extracted in 0.01 M sodium and potassium phosphate buffer (pH 7) and 0.1% sodium sulfite, which was rubbed onto the leaves of test plants previously dusted with 500-mesh Carborundum powder. Inoculated plants were kept under greenhouse conditions for 4–5 wk and monitored for symptom development.

Aphid transmission. Two species of aphids (*Myzus persicae* Sulz. and *Rhopalosiphum maidis* Fitch) were tested for their ability to transmit the virus from elephant grass. After a 30-min fasting period, aphids were given an acquisition feeding period of 30 min on infected elephant grass and were then transferred to healthy test plants for an additional 30 min. The aphids were then killed with a mevinphos spray. A total of 10 trials (five plants per trial) were made with each aphid species, and in each trial 10 aphids per plant were used.

Purification. The virus was purified according to the protocol of Marinho and Kitajima (16). Essentially, tissue was extracted with 0.5 M phosphate buffer containing 0.75% sodium sulfite and 0.01 M EDTA and clarified with 8% *n*-butanol, and the virus was precipitated with 8% PEG-6000. Precipitated virus was resuspended in 0.01 M borate buffer, pH 8.3, with 0.001 M EDTA and then centrifuged at 1,500 *g* at 4 C for 10 min. The supernatant was centrifuged at 100,000 *g* at 4 C for 50 min, and the pellet was resuspended in 5 ml of the borate/EDTA buffer and centrifuged at 2,000 *g* at 4 C for 10 min. The virus was further purified by cesium chloride isopycnic centrifugation, and the visible

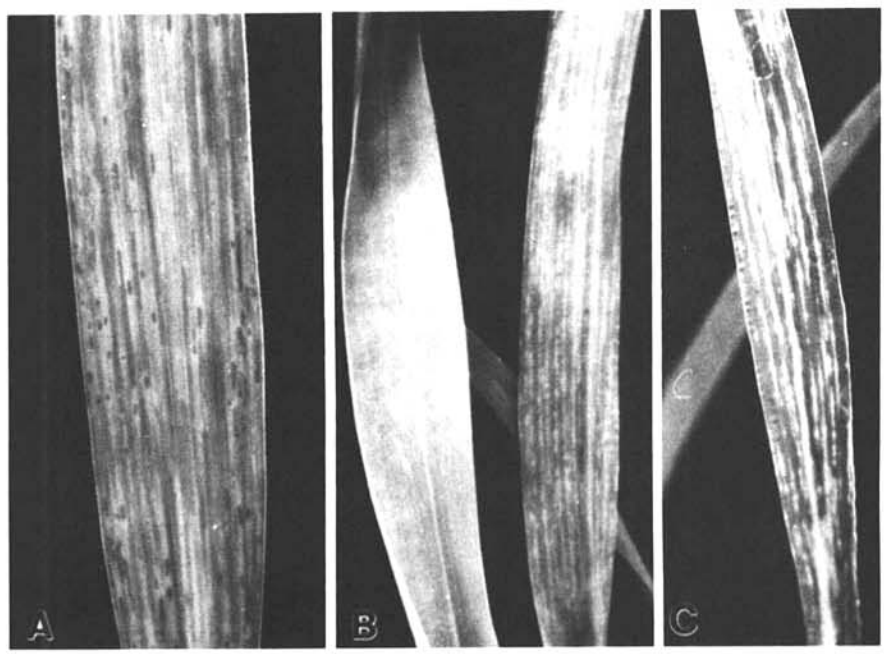

Fig. 1. (A) Naturally infected elephant grass (*Pennisetum purpureum*). **(B)** Corn (*Zea mays*) and **(C)** sorghum (*Sorghum bicolor*) infected experimentally with the virus isolated from elephant grass.

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band was collected, diluted in borate/EDTA buffer, submitted to a final high-speed centrifugation at 100,000 *g* for 50 min, and resuspended in the borate buffer without EDTA. The purity and concentration of the preparations were determined by spectrophotometry (extinction coefficient of 2.5 mg/ml/cm [10]) and electron microscopy.

Serology. Samples (1 mg) of purified virus preparation were emulsified with equal volumes of Freund's adjuvant and injected intramuscularly in a rabbit. After 3 injections at weekly intervals, the rabbit was bled and the serum evaluated by microprecipitin test. Gammaglobulin was partially purified by saturated ammonium sulphate and conjugated to alkaline phosphatase for direct enzyme-linked immunosorbent assay (4). Serological relationships between the virus isolated from elephant grass and other potyviruses were determined by agar gel double-diffusion test (15,17) and immunoelectron microscopy (9). Antisera to the following viruses were used: barley yellow mosaic, guinea grass mosaic, rice necrosis, wheat streak mosaic, sugarcane mosaic, turnip mosaic, maize dwarf mosaic, cowpea rugose mosaic, tradescantia mosaic, canavalia mosaic, soybean mosaic, western celery mosaic, onion yellow dwarf mosaic, bidens mottle, passionfruit woodiness, watermelon mosaic 2, bean common mosaic, peanut stripe, peanut mottle, and carnation vein mottle.

Electron microscopy. Examination of either crude or purified virus preparations was made by negative staining in 1% sodium silicotungstate. Particle length was determined in leaf-dip preparations using potato virus S particles as a standard (650 nm). About 100 particles of each virus were measured to estimate the modal length.

Leaf tissues from either healthy or diseased plants (elephant grass, corn, and sorghum) were fixed in a modified Karnovsky solution (2% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer), postfixed with 1% osmium tetroxide, stained en bloc with 0.5% aqueous uranyl acetate, dehydrated in acetone, and embedded in Spurr's low-viscosity medium. Thin sections were obtained in a microtome equipped with a diamond knife, then stained with uranyl acetate and lead citrate.

The procedure by van Lent et al (24) was used to detect viral antigens in situ by immunogold labeling in thin sections of aldehyde-fixed tissues embedded in LR gold medium. Antibody was diluted (1:2,000), and protein A-colloidal gold of 7 nm was used for labeling.

Chemical analysis. The virus was disrupted and analyzed in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (14). The method of Dodds et al (5) was used to try to isolate double-stranded RNA (dsRNA) associated with the infection.

RESULTS

Transmission tests and host range. The virus from elephant grass was mechanically transmitted to a few varieties of corn (Lili, Bol.III Moroti 32, Caingang Composto, CE 1, Composto Cruz Cuzco, Composto Racial Dentado Branco, Composto Jaiba, and Kalahary Blitz) and sorghum (CMSX 350, BR 005R, CMSXS 182, BR 505, and BR 400). Varieties of both corn and sorghum developed chlorotic spots and streaks 1–2 wk after inoculation (Fig. 1B and C). Infection by the same virus from elephant grass was confirmed by electron microscopy examination and serological tests (serologically specific electron microscopy, double diffusion, and enzyme-linked immunosorbent assay [ELISA]). Despite exhaustive attempts using various buffers and different plant ages and varieties, the virus was not mechanically transmitted to elephant grass. The grafting of infected elephant grass stems into healthy stems was also an unsuccessful method of transmitting the virus.

Aphid transmission tests. The virus was not transmitted from elephant grass to corn (BR 400 and Lili), sorghum (CMSX 350), wheat (Candeias and BR 16), oat, or elephant grass by either *M. persicae* or *R. maidis*.

Purification and serology. The virus from elephant grass was readily purified, and yields of approximately 8 mg/100 g of leaf tissue was obtained. When examined in the electron microscope, only filamentous particles (Fig. 2) similar to those in leaf-dip preparations (Fig. 3A) were visible. A highly specific antiserum with a titer of 1:4,096 in microprecipitin tests was obtained from rabbits immunized with the purified virus preparation. The antiserum did not react with sap from healthy elephant grass. In direct ELISA, the antiserum reacted up to dilution of 1:12,800.

None of the antisera against 20 different potyviruses reacted with the elephant grass virus in agar gel double-diffusion tests or in serologically specific electron microscopy, but a typical decoration occurred when homologous antiserum was used (Fig. 3B).

Electron microscopy. Leaf-dip preparations from naturally infected elephant grass or mechanically infected corn and sorghum contained elongated particles with a modal length of 730 nm (Fig. 3A). In thin sections of infected leaves from elephant grass, corn, and sorghum, a large amount of lamellar inclusions (Fig. 4A), typical of potyviruses included in subdivision I of Edwardson's classification, were found. Electron-dense tubular material of unknown nature, and so far undescribed for other potyviruses (J. R. Edwardson, *personal communication*), was also present in the cytoplasm. Also, fine filaments always present in the cytoplasm background (Fig. 4A, arrows) reacted with antibodies against elephant grass mosaic virus (EGMV) in immunolabeling tests (Fig. 4B).

Chemical analysis. In SDS-PAGE of virus preparations, a single peptide of 36 kDa (Fig. 5) was obtained. Although viral RNA could not be obtained, a dsRNA of 6.7 mDa was extracted from infected elephant grass tissues (Fig. 6).

DISCUSSION

The results obtained indicated that the elephant grass with mosaic symptoms was infected by a virus. This virus appears to have a very restricted host range

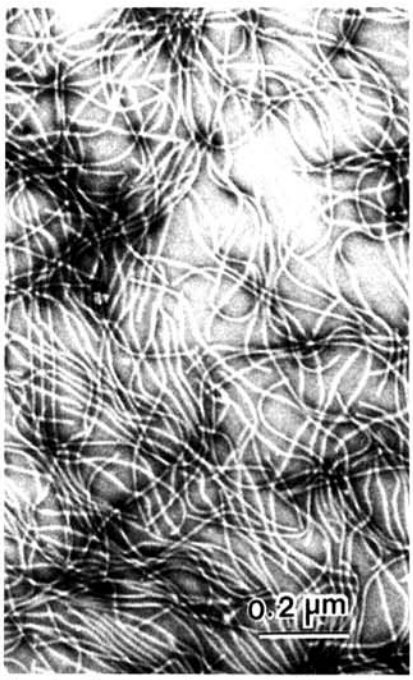

Fig. 2. Electron micrograph of negatively stained particles of the virus from elephant grass, in a purified preparation.

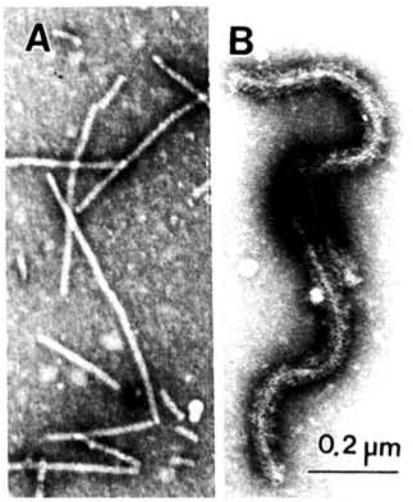

Fig. 3. (A) Elongated particles in a leaf-dip preparation, which become decorated **(B)** when treated with anti-elephant grass virus immunoglobulins.

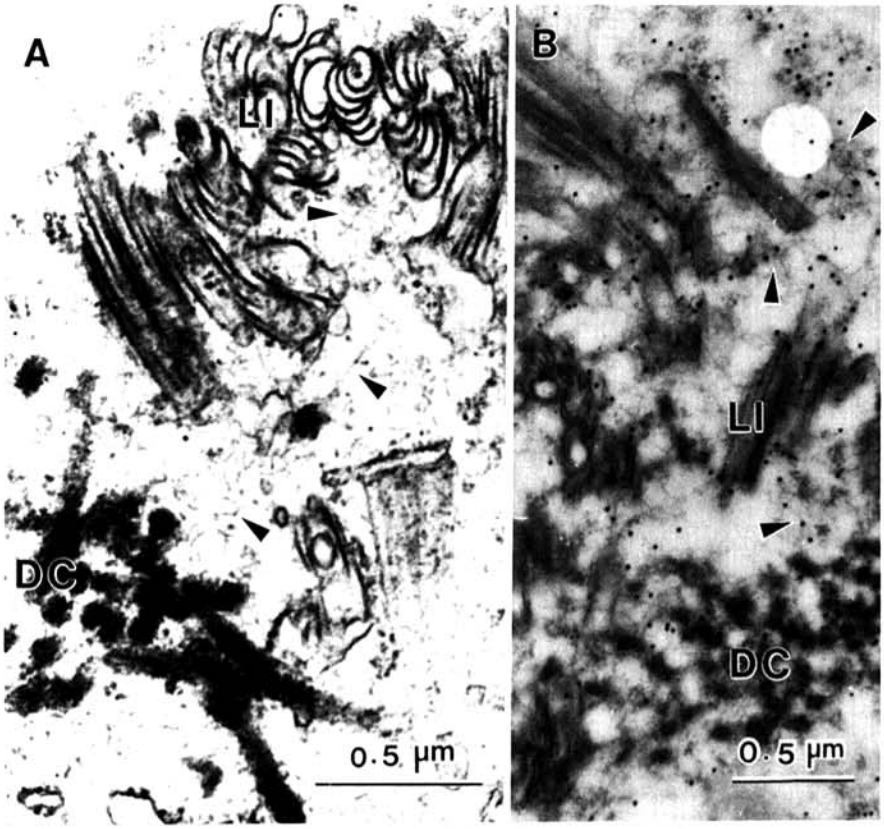

Fig. 4. Thin section from infected leaf tissues of infected elephant grass. **(A)** Part of a mesophyl cell, with lamellar inclusions (LI), virus particles (arrowheads), and dense cylinders (DC). **(B)** Corn leaf cell immunogold-labeled with anti-elephant grass virus immunoglobulin. Note that gold particles appear mostly on the filamentous particles. Lamellar inclusions and dense cylinders are not specifically labeled.

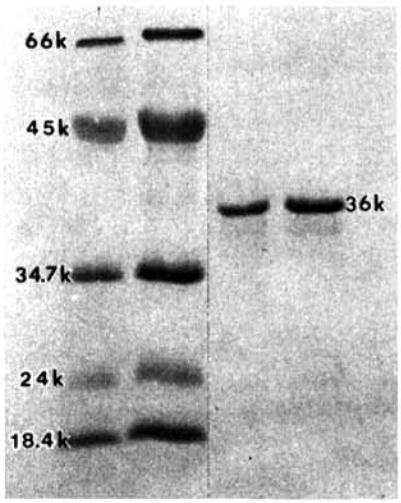

Fig. 5. SDS-PAGE profile of the coat protein of the elephant grass virus. Markers are bovine plasma albumin (66kDa), egg albumin (45 kDa), pepsin (34.7 kDa), trypsinogen (24 kDa), and β-lactoglobulin (18.4 kDa) (lanes A and B); and elephant grass virus capsid protein of 36 kDa (lanes C and D).

and was not transmitted to the original host species. Nevertheless, we believe that it is the causal agent of the mosaic in elephant grass and propose that the name *elephant grass mosaic virus* (EGMV) be used until the virus is further characterized. The virus may be a potyvirus, but it differs in host range, insect transmission, and serological relationships from known potyviruses that naturally infect Gramineae (1,3,8,11,12, 18–23). Edwardson and Christie (6), in their extensive review on potyviruses, refer only to experimental infection of elephant grass by sugarcane mosaic virus (SCMV), which does not share a similar host range nor serological relationship with EGMV.

The particle morphology and cytopathic effects of EGMV are similar to those of potyviruses (7,10). Serology and immunolabeling research indicated that the symptoms in corn and sorghum were due to the same virus isolated from elephant grass. The size of EGMV capsid peptide is within the range of known potyvirus coat proteins, and although we could not isolate the nucleic acid from the EGMV, the size of the dsRNA extracted from infected leaf tissue suggests that the viral genome is an RNA of about 10 kb, similar to other potyviruses (7,23).

Additional work is required to definitely characterize EGMV as a potyvirus. This includes, at least, characterization of the nucleic acid, determining the mode of transmission, and serological assays with the antiserum to EGMV with other viruses. Although EGMV has been found only in elephant grass, experimental transmission to corn and sorghum indicates that the virus maybe of economic importance.

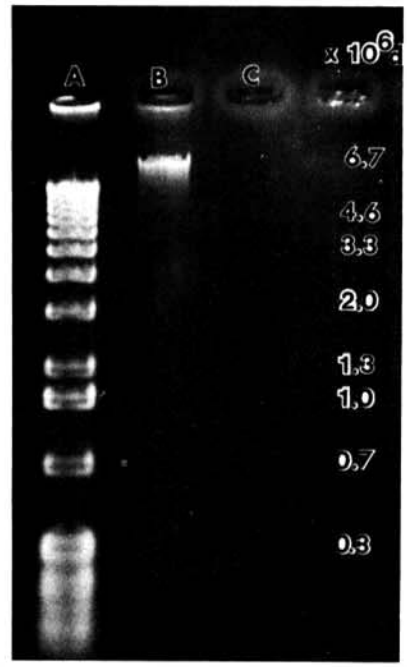

Fig. 6. Agarose gel electrophoresis pattern of the double-stranded RNA from infected elephant grass leaf extract. Marker DNA ladder (lane A); leaf extracts from infected (lane B) and healthy (lane C) elephant grass plants.

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