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Reprinted from August 1976 PHYTOPATHOLOGY, Volume 66, No. 8
Published by The American Phytopathological Society, Inc.
3340 Pilot Knob Road, St. Paul, Minnesota 55121

Guinea Grass Mosaic Virus, a New Member of the Potato Virus Y Group

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The authors are indebted to R. B. Bartels (Braunschweig, W. Germany), D. A. Govier and R. W. Gibson (Rothamsted, England), J. J. de Wijs (Abidjan, Ivory Coast), T. P. Pirone (University of Kentucky, USA), and L. Bos (Wageningen, The Netherlands) for providing viruses and antisera. We wish to thank L. Hirth for constructive criticism of the manuscript and Mrs. C. Dasen for helping with the English translation.

Accepted for publication 10 February 1976.

ABSTRACT

THOUVENEL, J. C., L. GIVORD, and P. PFEIFFER. 1976. Guinea grass mosaic virus, a new member of the potato virus Y group. Phytopathology 66: 954-957

A mechanically transmissible virus, which infects only graminaceous plants, caused mosaic on Guinea grass *(Panicum maximum)* in the Ivory Coast. The virus particles are flexuous rods 800 nm long. The virus is related serologically to pepper veinal mottle virus from the Ivory Coast and Ghana and is probably a new member of the potato virus Y group. It is named Guinea grass mosaic virus. A purification method using Triton X-100 as a clarifying agent is described.

Additional key words: Panicum, potyviruses, grass viruses.

Guinea grass (*Panicum maximum* Jacq.) is a wild graminaceous plant, which is widespread in all tropical and subtropical regions. Its high yields and dry-matter content make it a good fodder plant. This grass is grown at the ORSTOM center of Adiopodoumé (Ivory Coast) for genetic researches. Some of the new cultivars introduced from East Africa in 1967 showed mosaic symptoms a year later. The symptoms had not been seen previously in the collection. Susceptible plants were easily spotted in the collection because cultivars are propagated by cuttings year after year. *Panicum maximum* cultivars K 189b and K 211 were particularly susceptible, the former showing a light-green mosaic and the latter a yellow mosaic. The virus was mechanically transmissible from Guinea grass to healthy seedlings of Guinea grass and of some other graminaceous plants, but failed to infect plants from other families.

Since "panicum mosaic virus" infects *Panicum virgatum* in the USA (8, 11, 12), we shall refer to the virus described here as Guinea grass mosaic virus (GGMV).

MATERIALS AND METHODS

Virus and plant sources.—The GGMV inoculum for all tests was prepared from *P. maximum* cultivar K 189 b plants that were naturally infected for several years. *Setaria italica* Beauv. and *P. maximum* were used for virus multiplication, for purification, and as assay plants for all tests.

For host-range studies, at least 20 seedlings of each species or cultivar were inoculated at the two- to three-leaf stage, either with purified virus or with crude sap in 0.01 M potassium phosphate buffer pH 7.5 containing 0.02 M cysteine hydrochloride (1 g tissue to 2 ml buffer). Inoculations back to *S. italica* were made with all plants as a routine check and to detect symptomless infections. Clarified sap of infected or apparently healthy plants was tested against GGMV antiserum in a microprecipitation reaction.

Determination of in vitro properties.—Crude sap diluted with the previously described phosphate buffer was used for the dilution end-point studies. The thermal inactivation point was tested by immersing 1-cm diameter Pyrex tubes containing 2-ml samples of sap in a waterbath at a given temperature for 10 minutes and then in an icebath. To test in vitro aging, crude sap kept at 24 C and 4 C, as well as whole diseased leaves kept at 4 C, were assayed every day.

Virus purification and density-gradient centrifugation.—Systemically infected leaves of *P. maximum* harvested from the collection were used. In some instances the virus was extracted from mechanically infected plants.

Purification of the virus was achieved by a combination of the methods described by Damirdagh and Shepherd (4) and van Oosten (9): Mince 400 g of leaves with 400 ml 0.5 M K_2HPO_4 buffer pH 7.5, containing 1% sodium bisulfite and 1 M urea (15). Centrifuge expressed sap at 10,500 *g*. Add 5% (v/v) Triton X-100 and stir for 0.5 hours. Centrifuge at 78,480 *g* for 2 hours. Resuspend the pellet overnight in 40-50 ml of 0.05 M sodium borate buffer pH 8.2, containing 1 M urea and 0.1% mercaptoethanol. Centrifuge at 6,000 *g*. Sediment the supernatant through 8 ml of 20% sucrose in a SW 25.1 rotor for 2 hours at 51,200 *g*. Resuspend the pellet in 2 ml of the previously described borate buffer for at least 2 hours and centrifuge at 6,000 *g*. Purify the virus further by density-gradient centrifugation in 10-40% sucrose for 2 hours at 51,500 *g*. Remove virus band, dilute three times in 0.05 M sodium

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borate buffer pH 8.2, and concentrate by centrifugation at 78,480 *g* for 2.5 hours.

Gradients were collected by pushing up the tube contents with a 50% sucrose solution. The optical density was recorded in the 3-mm flow cell of a Uvicord LKB spectrophotometer.

Ultraviolet absorption spectra were determined with a Zeiss PMQ II spectrophotometer in a 1-cm cell.

Electron microscopy.—Purified virus was stained with 0.5% uranyl acetate in 0.05% ammonium acetate buffer pH 7, containing 0.2% EDTA. Micrographs were taken with a Siemens Elmiskop 1-A at ×20,000. Length measurements were made on prints with a final magnification of ×60,000.

Insect transmission.—The aphids *Rhopalosiphum maidis* Fitch. and *Hysteroneura setariae* Thomas were used in transmission tests with GGMV, since *P. maximum* and *S. italica* are good host plants for these two aphid species (5). *Aphis gossypii* Glov. and *Aphis spiricola* Patch. also were tested, but they do not multiply on graminaceous plants. These four species of Aphididae are known to be potyvirus vectors.

After an acquisition period of 10 days on diseased *S. italica*, the insects were transferred to healthy young *S. italica* plants for an indefinite inoculation time.

Serology.—Antiserum to GGMV was prepared in rabbits. Six weekly injections were given. We used a virus preparation purified by the method described above. The preparation contained 7-8 OD_{260nm} units of virus per ml emulsified with an equal volume of Freund's incomplete adjuvant (Difco). The microprecipitation reaction under paraffin oil in Petri dishes was used (13). For antigen preparation, plant juice was clarified with an equal volume of chloroform.

RESULTS

Symptomatology.—*P. maximum* plants infected with GGMV under natural conditions showed general dwarfing and a light-green mosaic. On some cultivars, or when the plants were young, the first symptom was a light-green eyespot. One cultivar with drier leaves showed a more elongated, spindle-shaped eyespot. Later on, the eyespots anastomosed to form a striped mosaic (Fig. 1 and 2). Symptoms on mechanically inoculated plants appeared 1 week after inoculation and were identical to those described above.

Host range.—A few hosts were infected with GGMV by mechanical inoculation. Spots and striped mosaic occurred in the following Gramineae: *Brachiaria deflexa, Bromus arvensis, B. commutatus, B. macrostachys, B. racemosus, B. sterilis, Coix lacryma-jobi, Echinochloa crus-galli, Oplismenus hirtelus, Panicum bulbosum, P. crus-galli, P. maximum, P. miliaceum, Paspalum racemosum, Setaria glauca, S. italica, S. macrochaeta, S. verticillata, Stenotophrum secundatum,* and *Zea mais.*

The virus was not, however, recovered from the following inoculated Gramineae: *Agropyron junceiforme, Avena fatua, A. paniculata, A. strigosa, Bromus erectus, B. inermis, B. unioloides; Dactylis glomerata, Digitaria sanguinalis, Eleusine coracana, E. indica, E. tocussa, Hordeum murinum, H. vulgare, Lolium multiflorum, L. rigidum, Miscanthus sinensis, Oryza sativa, Panicum capillare, Paspalum distychum, P. virgatum, Pennisetum japonicum, Poa pratensis, Sorghum vulgare, Trisetum flavescens, Triticum aestivum* and *T. durum.*

None of the following 22 species, which represent seven families, could be infected with GGMV: Amaranthaceae–*Gomphrena globosa;* Chenopodiaceae–*Chenopodium amaranticolor, C. quinoa, Spinacia oleracea;* Compositae–*Helianthus annuus;* Leguminosae–*Phaseolus pinto, P. vulgaris* 'Prince', *Pisum sativum* 'Alaska', *Vicia faba, Vigna cylindrica, V. sinensis;* Malvaceae–*Hibiscus esculentus;* Rutaceae–*Ruta graveolens;* Solanaceae–*Capsicum annuum, C. frutescens, Datura stramonium, Hyosciamus niger, Lycopersicon esculentum, Nicotiana clevelandii, N. megalosiphon, N. tabacum* 'Samsun', *Solanum melongenum.*

In summary, the host range of GGMV seems to be restricted to graminaceous plants and especially to the

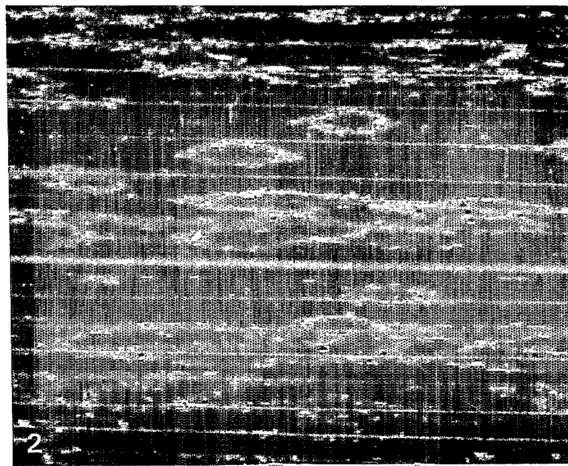

Fig. 1-2. 1) Middle and right rows, naturally infected *Panicum maximum* 'K 189b' in the collection. Left row, cultivar tolerant to Guinea grass mosaic virus. **2)** Detail of an infected *P. maximum* 'K 189b' leaf showing the characteristic eyespots.

Paniceae, Maydeae, and Bromeae tribes.

In vitro properties.—Plants inoculated with dilutions up to 10^{-2} became systemically infected; no infection occurred at a dilution of 10^{-3}. The virus still was infectious after heating for 10 minutes at 45 C, but not at 50 C. Virus stored at 24 C lost infectivity within a few hours; it remained infectious at 4 C for 24 hours. Diseased leaves stored at 4 C were still infectious after 4 days. Frozen diseased leaves of Guinea grass remained infectious for 2 weeks, but not more. If they were frozen and thawed many times, they rapidly became noninfectious.

Transmission.—No transmission could be obtained with aphids. Dodder did not developed on Guinea grass or other graminaceous plants. In the fields, *P. maximum* plants were multiplied by cuttings and the virus thus was propagated easily. Seed transmission never was observed in Guinea grass or in 500 seedlings raised in the greenhouse from seeds from diseased *S. italica* plants. After 1 month, none of the seedlings showed symptoms and no virus was recovered from them.

Density-gradient centrifugation.—At the end of the density-gradient centrifugation, a single opalescent band was seen at 24-25 mm beneath the meniscus. The UV spectrum of this material was typical of a nucleoprotein, with a maximum at 260 nm and a minimum at 247 nm. The maximum/minimum absorbance ratio was 1.09 to 1.12. The 260/280 absorbance ratio ranged from 1.20 to 1.24 (without correction for light scattering) and indicated a nucleic acid content of 6.0 to 6.5%. Purification yields were calculated by the same method as for PVMV (1) and ranged from 10-20 mg of virus per kilogram of fresh leaves.

Electron microscopy.—GGMV particles appeared in the electron microscope as flexuous rods of about 15-nm diameter. Of 213 particles measured, 85 (40%) were 816 ± 16 nm long (Fig. 3-a). The particles of purified GGMV became straight upon exposure to 0.05 M $MgCl_2$ (Fig. 3-b), a feature characteristic of the potyvirus group (6).

Serology.—Purified GGMV reacted with homologous antiserum up to a dilution of 1/2,048. A positive reaction was obtained between this antiserum and pepper veinal mottle virus from Ivory Coast (PVMV-CI) up to a dilution of 1/128. When GGMV was tested against PVMV Ghana strain (PVMV-Gh) and PVMV-CI antiserum, it reacted up to a serum dilution of 1/32 and 1/64, respectively. There was no positive reaction between GGMV antigen and the antisera against henbane mosaic virus (titer 8,000), potato virus A (titer 4,096), tobacco etch virus (titer 512), Colombian datura virus (titer 2,048), sugarcane mosaic virus strains H and J (titer 100), and passiflora ringspot virus (titer 4,096).

Further, henbane mosaic virus, cocksfoot streak virus, ryegrass mosaic virus, and Passiflora ringspot virus failed to react with antiserum against GGMV. Finally, GGMV antiserum showed no positive reaction with Panicum mosaic virus (PMV), which indicated that PMV and GGMV are two different viruses.

DISCUSSION

The partially characterized Panicum mosaic virus (PMV) (11, 12) infects Paniceae plants as does GGMV, but also multiplies in *Digitaria sanguinalis,* which is not a host for GGMV. Further, GGMV antiserum fails to react with PMV antigen. Lee and Toler (7) found a strong serological relationship between the icosahedral Saint Augustine decline virus and PMV, and more recent studies definitely have identified PMV as an isometric virus (8). Thus, the only common feature of these two viruses is their host range.

When exposed to Mg^{++} ions, the particles of GGMV become straight, as do those of bean yellow mosaic, henbane mosaic, and pepper veinal mottle viruses (6). This Mg-induced morphological change, together with the size and morphology of the particles, suggests that GGMV is a member of the potyvirus group, although transmission by aphids has not yet been achieved. The inclusion of GGMV in the potyvirus group is further confirmed by its serological relationship with PVMV-Gh and PVMV-CI.

The potyviruses that can infect graminaceous plants are: anthoxanthum mosaic virus (2), cocksfoot streak

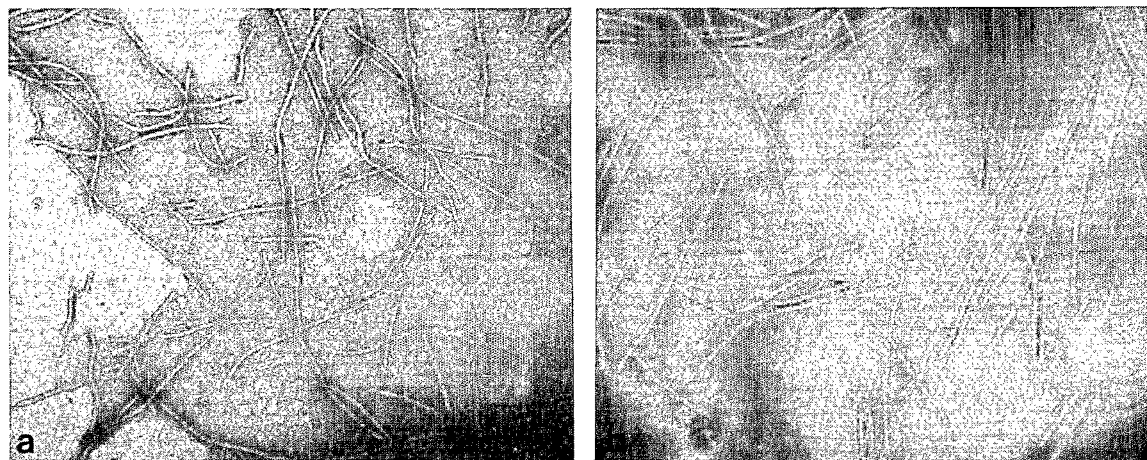

Fig. 3-(a, b). a) Electron microscopy of purified Guinea grass mosaic virus; **b)** the same, but incubated in 0.05 M $MgCl_2$. Magnification: × 38,500.

virus (3), sugarcane mosaic virus (SCMV) and its strains, maize dwarf mosaic, abaca mosaic, and sorghum red strip (10). Anthoxanthum mosaic and cocksfoot streak viruses differed from GGMV by their host range. Moreover, the serological tests between GGMV and cocksfoot streak virus were negative. SCMV infected the same tribes as GGMV, but its host range is more extended (14). For example, GGMV did not develop in *Eleusine coracana, Miscanthus sinensis, Oryza sativa,* and *Panicum capillare*. Further, plants highly susceptible to GGMV were not easily infected with SCMV. SCMV and its strains were very difficult to purify and had low antigen titers (256), whereas GGMV gave high titers (2,048). Finally, we found no relationship between antiserum against SCMV and its strains, and GGMV antigen. Consequently, we conclude that GGMV is not a strain of SCMV and is a new member of the potyvirus group.

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