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A Strain of Guinea Grass Mosaic Virus from Pearl Millet in the Ivory Coast

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With 6 figures

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Abstract

A virus disease of pearl millet (*Pennisetum americanum*) in the Ivory Coast has symptoms consisting of a light-green mosaic of variable severity, followed by dwarfing. The causal virus is mechanically transmissible and aphid-borne, but not seed-borne. It was purified, has flexuous filamentous particles about 820 nm long, and is a member of the potyvirus group. Its host range, and biological, physico-chemical and serological properties indicate that it is a strain of guinea grass mosaic virus.

Zusammenfassung

Ein Stamm des Guinea Gras Mosaik Virus von Perlhirse in der Elfenbeinküste

Eine Viruserkrankung der Perlhirse (*Pennisetum americanum*) in der Elfenbeinküste hat Symptome, die aus einem hellgrünen Mosaik unterschiedlicher Schwere bestehen, gefolgt von Zwergwuchs. Das ursächliche Virus ist mechanisch und von Läusen übertragbar, nicht jedoch samenübertragbar. Es wurde gereinigt, besitzt gedreht filamentöse etwa 820 nm lange Partikel und ist Mitglied der Potyvirus-Gruppe. Sein Wirtsspektrum, seine biologischen, physikalisch-chemischen und serologischen Eigenschaften weisen darauf hin, daß es ein Stamm des Guinea Gras Mosaik-Virus ist.

Pearl millet (*Pennisetum americanum*) is an important crop in sahelian and sub-sahelian countries. It is grown in the northern part of the Ivory Coast and is the subject of a crop improvement programme. To this end, efforts are

being made to find cultivars resistant to diseases and to insect pests and this necessitates research to identify the causes of virus-like disease occurring in the region.

In two experimental areas, one near Adiopodoumé (South) and the other near Ferkessedougou (North) plants showing mosaic symptoms were observed and were suspected to be affected by a virus disease. We describe here this disease and its causal agent.

Materials and Methods

Virus isolate

The virus was isolated from *P. americanum* grown at the ORSTOM Centre of Adiopodoumé (Ivory Coast). A stock isolate cultured in *P. americanum* provided inoculum for all the tests.

Host range tests

For host range studies, at least 20 seedlings of each species at the 2—3 leaf stage were mechanically inoculated either with purified virus, or with crude sap diluted (5 to 10 ml per g leaf) in 0.1 M potassium phosphate buffer, pH 7.1, containing 0.02 M cysteine hydrochloride and 0.02 % bentonite. Back inoculations were made on *P. americanum* to detect symptomless infection. Serological tests were also done using crude sap clarified by treatment with chloroform (v/v).

Aphid transmission

Aphids (*Hysteroneura setariae* Thomas or *Rhopalosiphum maidis* Fitch) cultured on healthy *Setaria italica* and on healthy *Zea mays* were starved for 1 or 2 h, then allowed to feed on young diseased leaves of pearl millet for 5—10 min before being transferred to healthy seedlings (10 aphids per seedling), during 2 days. Then they were killed by insecticide.

Determination of properties *in vitro*

Crude sap diluted in 0.1 M phosphate buffer pH 7.1, was used for the studies of the following properties: dilution end-point, thermal inactivation point, and longevity *in vitro* at 24 °C, 4 °C and —20 °C. The methods described by NOORDAM (1973) were used.

Virus purification

Virus particles were purified as described by THOUVENEL *et al.* (1978) for GGMV-A and LAMY *et al.* (1979) for GGMV-B. However in some experiments the virus containing pellets were resuspended in 0.01 M potassium phosphate buffer, pH 8.4.

UV absorption spectrum

Ultraviolet absorption spectra of purified virus preparation were determined with a Beckman-U.V.-5230 spectrophotometer. The correction for light-scattering was obtained by the graphical method described by NOORDAM (1973).

Isoelectric point

The isoelectric point of the virus particles was determined as described by LAMY *et al.* (1979) for the GGMV-B. The optical density (OD 260 nm) and pH values of the fractions were respectively recorded with the 3 mm flow cell of an LKB Uvicord absorptiometer and an ORION 701 A pH-meter.

Polyacrylamide gel electrophoresis of virus coat protein

The molecular weight of the virus coat protein was determined by the method of WEBER and OSBORN (1969) using gels containing 5 % to 12 % acrylamide, and sodium dodecyl sulphate as described by VAN REGENMORTEL *et al.* (1972).

Electron microscopy

To examine the morphology of virus particles purified preparations, or crude sap clarified with chloroform, were stained with 2 % uranyl acetate for 1 min. Micrographs were taken with the Siemens Elmiskop 102 of the GERME (Groupement d'Etude et de Recherche en Microscopie Electronique) at Adiopodoumé (Ivory Coast). Length measurements were made on photographs with a final magnification of ×90,000. To study the cytophathological aspects of the virus, pieces of leaf tissues were processed as described by MORGAN and ROSE (1967) and were sectioned with a Sorvall MT_2-B ultra Microtome. Sections were stained by floating the grids on 3 % uranyl acetate for 10—15 min and then post-stained with lead citrate (REYNOLDS 1963) for 3 min before examination in the electron microscope.

Serology

An antiserum to pearl millet mosaic was prepared by injecting a rabbit with 1 mg of purified particles of pearl millet mosaic virus in 1 ml, once a week for 5 weeks (NOORDAM 1973). Microprecipitin droplet tests were performed under paraffin oil (VAN SLOGTEREN 1955).

Results

Host range and symptomatology

Young diseased *P. americanum* plants show lines of lightgreen eye spots or a pale green mosaic, depending on the cultivar. These symptoms develop into a striped mosaic by elongation and anastomosis of the eye-spots (Plate Fig. 2). Some *P. americanum* plants show severe symptoms with dwarfing (Plate Fig. 1). Mechanical transmission of the causal agent is easy, and symptoms appear about 8 or 10 days after inoculation of 2—3 leaf seedling with infective sap. The virus was also transmitted by mechanical inoculation to the following species of Graminae, in which it induced a light green mosaic: *Bromus commutatus, B. macrostachys, Panicum crusgalli, P. maximum* (K 187), *Sorghum aroundinaceum, Zea mays.*

No transmission was obtained to the following species: *Avena fatua, A. sativa* cv. Maris Tabard, *Briza maxima, Bromus uniloides, Dactylis glomerata, Digitaria sanguinalis, Eleusine coracana, E. tocussa, Oryza sativa* cv. IRAT 13 and Iguape Cateto, *Saccharum officinarum, Triticum durum* cv. Hardi.

Seed transmission

About 1000 seeds harvested from diseased pearl millet plants were sown but none of the 428 seedlings obtained showed symptoms of the disease, moreover negative back-inoculations exclude symptomless infection.

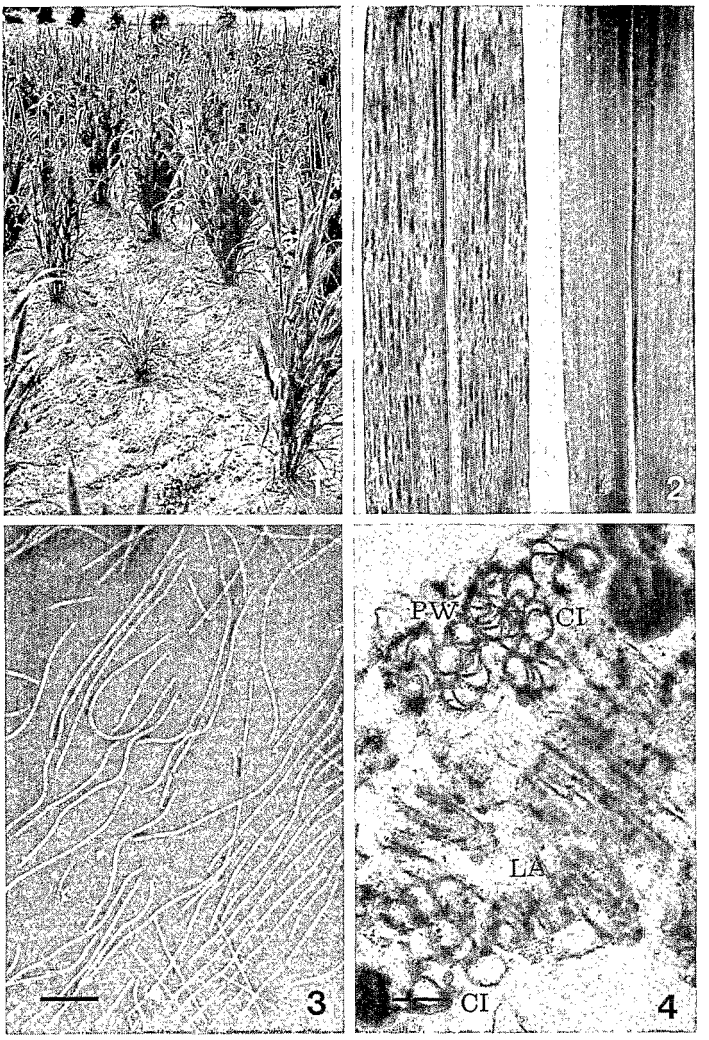

Fig. 1. Dwarfing induced by the pearl millet mosaic virus in *P. americanum*, observed in the field. • Fig. 2. Symptoms of pearl millet mosaic in (left) leaf of infected *P. americanum*; (right healthy leaf). Separate eye spots occur at the leaf margins, but have anastomosed at the middle. • Fig. 3. Electron micrograph of a purified preparation of pearl millet mosaic virus showing particles stained with 2 % uranyl acetate. (Bar represents 200 nm). • Fig. 4. Electron micrographs of virus-induced inclusions in *P. americanum* cells. LA = Laminate aggregates; CI = Cylindrical inclusions; CR = Circular inclusions; PW = Pinwheels. (Bar represents 300 nm)

Transmission by aphids

The virus was transmitted by *Hysteroneura setariae* to pearl millet seedlings. Four out of 19 seedlings showed the symptoms of the disease and serological tests revealed that additional three symptomless plants were infected. The virus was also transmitted from *P. americanum* to *Panicum maximum* by

Rhopalosiphum maidis. Six out of 20 seedlings showed pale symptoms of mosaic. Transmission following an acquisition access time of 5 to 10 min suggests it is of the non persistent type.

Properties *in vitro*

Crude sap was still infective after dilution to 10^{-2} but not to 10^{-3} and after heating for 10 min at 50 °C but not at 55 °C. Crude sap diluted to 10^{-1} remained infective for a few hours (less than 4 h in our experiments) at about 24 °C, but inoculum extracted from leaves previously stored 6 days at room temperature in a humid atmosphere was infective. Crude sap diluted to 10^{-1} was infective after 24 h but not 48 h at 4 °C, and was also infective after storage for 2 months at —20 °C. However, infectivity was abolished by repeated freezing and thawing.

Biophysical and biochemical properties

The UV absorption spectrum of purified suspension of virus particles (Text Fig. 1) shows an absorption maximum at 260 nm and a minimum at 247 nm. The $\frac{A_{260}}{A_{247}}$ ratio is 1.09 ± 0.03 and the $\frac{A_{280}}{A_{260}}$ ratio is 1.22 ± 0.02 (about 12 determinations) indicating a nucleic acid content of 6 % (LAYNE 1954). When corrected for light scattering, the absorption maximum is at 265 nm while the minimum stays at 247 nm (Text Fig. 1). The corrected ratios are: $\frac{A_{265}}{A_{247}} = 1.37$; $\frac{A_{260}}{A_{280}} = 1.15$.

When submitted to electrofocusing, the virus particles migrated as a single band at pH 4.7 ± 0.2. Three out of nine *P. americanum* plants developed mosaic symptoms after mechanical inoculation with the virus preparation which has been submitted to electrofocusing (3 experiments).

The apparent molecular weight of the virus coat protein, determined by polycrylamide gel electrophoresis (Text Fig. 2) was $32\,500 \pm 500$ d (average of 9 experiments) for both the pearlmillet mosaic virus and GGMV-A, and $34\,500 \pm 500$ d for GGMV-B (KUKLA 1981).

Electron microscopy studies

Virus preparations contained flexuous filaments particles (Plate Fig. 3) about 15 nm in diameter and 820 ± 10 nm in length (126 of the 258 particles measured) (Text Fig. 3).

Two types of inclusion were observed in ultrathin sections of diseased leaves, one with a circular and the other with a laminate structure. Using the nomenclature of EDWARDSON *et al.* (1968), the micrographs show cylindrical inclusions, pinwheels, circular inclusions, tubes and bundles (Plate Fig. 4).

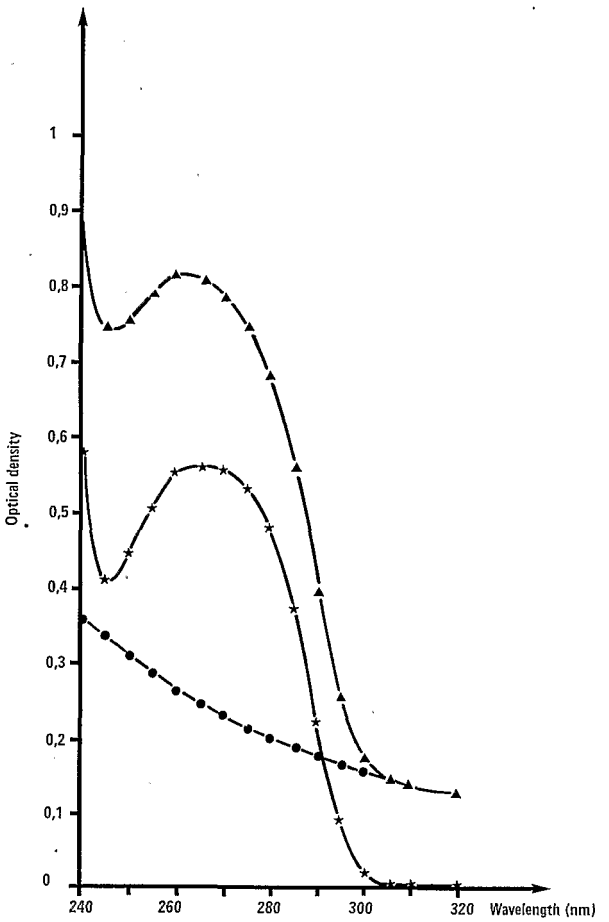

Fig. 1. UV absorption spectrum of a purified preparation of pearl millet mosaic virus before (—▲—▲—) and after (—✶—✶—) correction for light-scattering. The calculated correction (-●—●-) is also plotted.

Serology

Purified particles of the pearl millet mosaic virus reacted with homologous antiserum up to a dilution of 1/1024.

When tested against GGMV-A antiserum, homologous titre 1/2048 (THOUVENEL *et al.* 1976), the virus reacted up to a dilution of 1/1024 (SDI=1), and when tested against GGMV-B, antiserum homologous titre 1/1024 (LAMY *et al.* 1979), it reacted up to a dilution of 1/128 (SDI = 3). GGMV-A reacted up to a dilution of 1/512 (SDI = 1) and GGMV-B up to a dilution of 1/1024 (SDI=0) when tested against pearl millet mosaic virus antiserum (homologous titre 1/1024).

Pearl millet mosaic virus also tested for ability to react with antisera to several potyviruses (homologous titre in parentheses). Antiserum to pepper veinal mottle virus (1/8192) reacted up to a dilution of 1/256 (SDI = 5), and antisera to maize dwarf mosaic viruses A or B reacted at dilutions $< 1/8$:

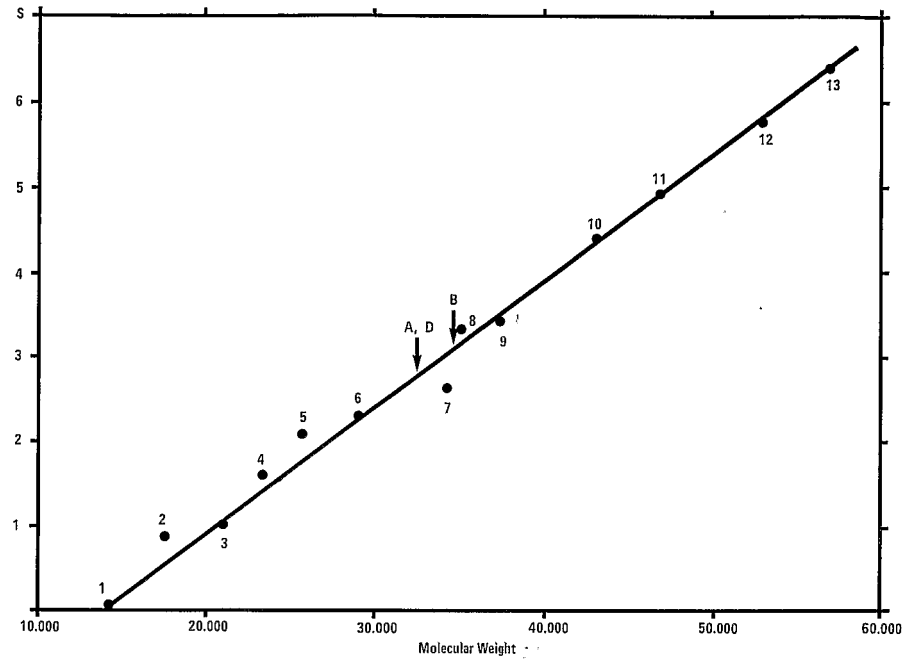

Fig. 2. Determination of the molecular weight of the coat protein of pearl millet mosaic virus from the plot of $S = \frac{\Delta \text{ Log RF}}{\Delta \text{ acrylamide concentration}}$ against the molecular weight. The markers, and their molecular weights in parenthesis are: 1. Lysozyme (14,000). 2. Tobacco mosaic virus coat protein (17,500). 3. Trypsin inhibitor from soybean (21,000). 4. Trypsin (23,300). 5. α-Chymotrypsinogen (25,700). 6. Carbonic anhydrase (29,000). 7. Carboxypeptidase (34,300). 8. Pepsin (35,000). 9. Alcohol dehydrogenase from yeast (37,500). 10. Ovalbumin (43,000). 11. 3-Phosphoglyceratekinase (47,000). 12. Glutamate dehydrogenase (53,000). 13. Pyruvate kinase (57,000)

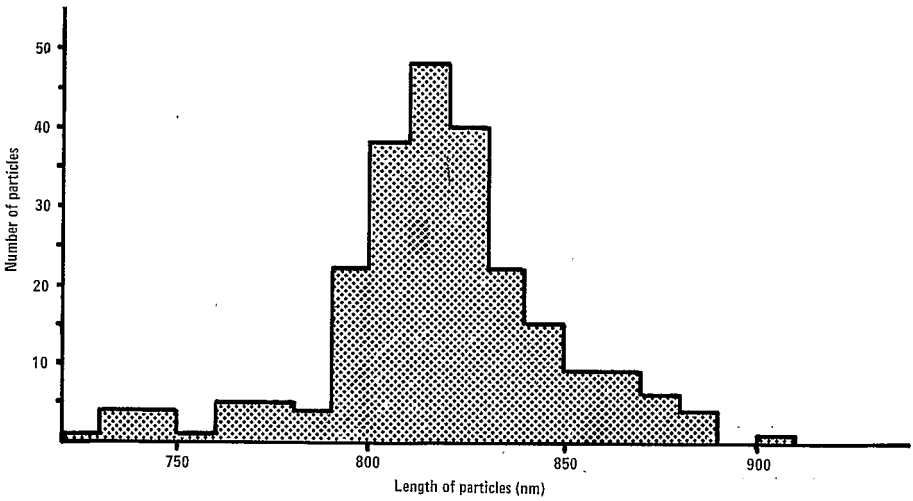

Fig. 3. Length distribution of particles from purified virus suspension observed by electron microscopy

The following antisera did not react with pearl-millet mosaic virus: bean common mosaic (1/512), bean yellow mosaic (1/1024), henbane mosaic (1/8192), passiflora ringspot (1/2048), peanut mottle (1/4096), potato A (1/160), potato Y (titre unknown), and sugarcane mosaic (titre unknown) virus antisera.

Discussion

Its biological and physico-chemical properties, the aphid transmissibility, inclusion bodies and serological properties indicate that pearl millet mosaic virus is a member of the potyvirus group. On the basis of the types of inclusions produced, pearl millet mosaic virus would be included in EDWARDSON's (1974) subdivision III of the potyvirus group, because all the characteristic inclusions (pinwheels, bundles, scrolls, tubes and laminated aggregates) typical of this subgroup, were observed. The serelogically related pepper veinal mottle virus also belongs to subdivision III (EDWARDSON 1974).

The properties (host-range, physico-chemical properties and serology) of pearl millet mosaic virus are, however, closest to those of guinea grass mosaic virus strain A (THOUVENEL *et al.* 1976) and strain B (LAMY *et al.* 1979). According to the host-range, among 21 gramineous species tested, 5 non-hosts and 5 hosts are common for the 3 viruses. Two species (*Bromus macrostachys* and *Panicum maximum*) which are host for GGMV-A and pearl-millet mosaic virus are non-hosts for GGMV-B. On the other hand 7 species which are non-hosts for GGMV-A and pearl millet mosaic virus are hosts for GGMV-B. Nevertheless two plants (*Bromus sterilis* and *Setaria italica*) are systemic hosts for GGMV-A and GGMV-B and negative hosts for pearl millet mosaic virus. Moreover we can notice that we have not found any common host for only GGMV-B and pearl millet mosaic virus. In this host-range composed of 21 gramineous species 7 are host for pearl-millet mosaic virus, 9 for GGMV-A and 13 for GGMV-B. Considering the natural hosts, the transmission is easiest and each virus causes strongest symptoms in its natural host than in the other. Pearl millet mosaic virus induces dwarfing in pearl millet and a mild mosaic in guinea grass and maize. GGMV-A can infect pearl millet and maize but with difficulty, and GGMV-B cannot infect guinea grass. For all these results we think that there is an adaptation to the natural host of each strain and we consider that pearl millet mosaic virus is closer to GGMV-A than GGMV-B.

The coat protein of pearl-millet mosaic virus has the same molecular weight as that of GGMV-A, but the degradation bands in gel electrophoresis are typically different for GGMV-A, GGMV-B and pearl millet mosaic virus. However an important difference between GGMV-A and pearl-millet mosaic virus is that only the pearl-millet virus could be transmitted by aphids. Whe therefore consider pearl millet mosaic virus to be an aphid-transmissible strain of guinea grass mosaic virus and we propose to name it GGMV-D.

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