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A strain of guinea grass mosaic virus naturally occurring on maize in the Ivory Coast

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SUMMARY

During a survey of cereal crops in Ivory Coast, a virus with flexuous filamentous particles *c.* 825 nm long was isolated from diseased maize. Its host range, biological properties, morphology and aphid transmission place it in the potyvirus group. Antigenically it is closely related to guinea grass mosaic virus, from which it differs in host range, aphid transmissibility and behaviour on isoelectric focusing. It is named guinea grass mosaic virus strain B.

INTRODUCTION

Maize (*Zea mays* L.) is now widely grown in the Ivory Coast and, as a twofold increase in production by 1985 is planned, special attention is now being paid to its pests and diseases. Several virus diseases have been detected during recent surveys (Lamy, Fauquet & Thouvenel, 1978); we here describe one which is induced by a potyvirus similar to, but distinguishable from, guinea grass mosaic virus (GGMV).

MATERIALS AND METHODS

The original virus isolate came from naturally infected maize, growing near Divo (South Ivory Coast), and was first transmitted mechanically to maize seedlings.

The maize virus was maintained and propagated on *Zea mays* cultivar IRAT CJB. For host range studies, young leaves of 25-day-old diseased maize plants were crushed in a mortar with 0·1 M potassium phosphate buffer (pH 7·1) containing 0·35% cysteine hydrochloride. The leaves to be inoculated were first dusted with Carborundum. At least 20 seedlings of each species were inoculated at the two- to three-leaf stage, and back-inoculations to maize were made routinely to detect symptomless infections. For determination of *in vitro* properties sap, obtained from the leaves of plants inoculated 15 days previously, was diluted with distilled water (1 g/10 ml). The thermal inactivation point was determined as described earlier (Thouvenel, Givord & Pfeiffer, 1976).

The maize virus and guinea grass mosaic virus were purified by the following method: mince 400 g of leaves with 1 litre of 0·5 M K_2HPO_4 buffer (pH 7·5) containing 1% sodium bisulphite and 1 M urea. Express sap through cheesecloth then add chloroform to 20%. Centrifuge at 6000 ***g*** for 5 min and then 2 h at 78 000 ***g***. Resuspend the pellets overnight in 10 ml of 0·05 M sodium borate buffer (pH 8·2), then centrifuge the suspension on a 20% sucrose cushion for 3 h at 78 000 ***g***. Allow the pellets to resuspend for 6 h and purify the virus further by density gradient centrifugation using a Beckman rotor SW27 for 2·5 h on columns of 10–40% (w/v) sucrose diluted in 0·05 M sodium borate buffer (pH 8·2). Remove the virus band, dilute with the above buffer and store at 4 °C.

The optical density of fractionated gradients was recorded using the 3 mm flow cell of a LKB Uvicord absorptiometer. Ultraviolet absorption spectra were determined with a Zeiss PMQ II spectrophotometer.

Purified virus preparations were mounted in 3% uranyl acetate, and micrographs were taken with a Philips EM 300 electron microscope at ×14 000. Length measurements were made from prints at a final magnification of ×40 000.

For insect transmission studies, aphids (probably *Rhopalosiphum maidis* Fitch) were starved for 1 h, then allowed to feed on young diseased leaves of maize for 3 min before transfer to healthy 10-day-old maize plants at the rate of 5 insects/plant. After 1 h the aphids were killed with insecticide.

Antiserum to the virus was prepared by injecting rabbits intramuscularly with 3 OD_{260nm} units of purified virus in 1 ml, emulsified with an equal volume of Freund's incomplete adjuvant (Difco), once a week for 4 wk. Serological tests were made by microprecipitation method under paraffin oil in Petri dishes (Slogteren, 1955); for antigens, purified virus suspensions were normally used at 0·3 OD_{260nm} units. For control tests, sap of healthy maize plants was submitted to the usual extraction procedure, except that the sedimentation on sucrose gradients was omitted.

Electro-focusing of virus particles was as follows: an LKB column, containing 110 ml ampholyte designated to give a gradient of pH 3·5–10 was filled with 3 OD_{260nm} units of purified virus, and a potential difference of 300 V applied during 12 h for establishing the pH gradient. The pH and optical density of the column contents were recorded with the flow cell of a Tacussel ISIS 20 000 pH-meter and the 3 mm flow cell of an LKB Uvicord absorptiometer.

RESULTS

Host range and symptomatology. Naturally infected *Zea mays* plants showed a slight green leaf mosaic (Plate fig. 1) and they were slightly stunted. Symptoms on old leaves were almost imperceptible. On young experimentally infected plants, light green spots appeared after inoculation on new leaves and finally coalesced to form a mild mosaic.

The following species in the Gramineae showed a light green leaf mosaic and yielded infective sap after inoculation with the maize virus:

Avena fatua, A. paniculata, A. strigosa, Bracharia deflexa, Bromus commutatus, B. racemosus, B. sterilis, Coix lacryma-jobi, Echinochloa crus-galli, Eleusine coracana, E. tocussa, Lolium multiflorum, Panicum bulbosum, P. capillare, Paspalum racemosum, Setaria glauca, S. italica, S. macrochaeta, S. verticilliata, Sorghum sudanense, S. vulgare and *Zea mays.*

No symptoms or symptomless infection was detected after inoculation of the following species (the number of plants tested of each species is given in parentheses):

Amaranthaceae: *Gomphrena globosa* (23). Cucurbitaceae: *Cucumis sativus* (10), Gramineae: *Bromus macrostachys* (28), *B. unioloides* (12), *Dactylis glomerata* (18), *Digitaria sanguinalis* (20), *Eleusine indica* (20), *Hordeum murinum* (27), *H. vulgare* (16), *Oryza sativa* 'IR 8' (30), *Panicum maximum* 'K 187' (90), *Paspalum distychum* (17), *Saccharum officinarum* (24). Leguminosae: *Vigna cylindrica* (14), *V. unguiculata* (15). Solanaceae: *Lycopersicon esculentum* (9), *Solanum melongena* (16).

Attempts failed to detect viral antigen with homologous antiserum in sap of inoculated *Panicum maximum* 'K 187'.

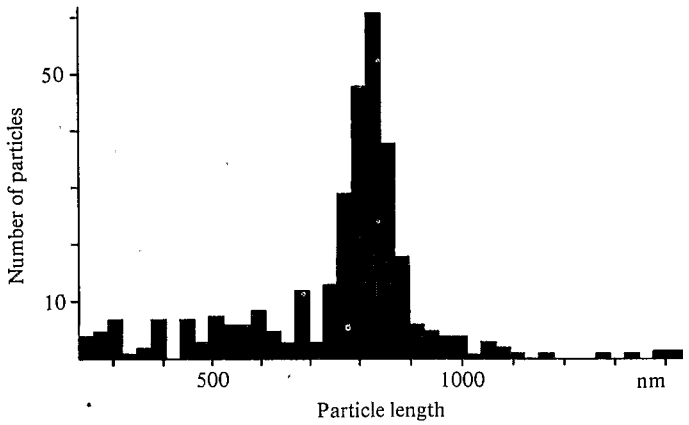
In vitro *properties.* Sap was infective after dilution to 10^{-2} but not 10^{-3}, after heating for 10 min at 50° but not 55 °C, and after storage at 24 °C for 5 but not 8 h, and at 4 °C for 3 but not 4 days. Dehydrated leaves remained infective for at least 3 months at 4 °C but infectivity of sap was abolished by freezing.

Ultraviolet absorption. The u.v. absorption spectrum of purified virus preparations showed a

maximum at 260 nm and a minimum at 248 nm. The $A_{260/248}$ ratio was $1{\cdot}09 \pm 0{\cdot}01$ and the $A_{260/280}$ ratio was $1{\cdot}22 \pm 0{\cdot}02$ indicating a nucleic acid content of about 6·0% (Layne, 1954).

Particle morphology. The virus has flexuous filamentous particles *c.* 15 nm in diameter (Plate, fig. 2). Of 326 particles measured, 62 (20%) were 825 ± 15 nm long (Text-fig. 1).

Behaviour on isoelectric focusing. When submitted to electrofocusing, the virus migrated to form a single band at pH $5{\cdot}3 \pm 0{\cdot}2$. In the same conditions, GGMV particles banded at pH $4{\cdot}7 \pm 0{\cdot}2$. Neither virus was infective after banding in the ampholine column and some of the particles were precipitated.



Text-fig. 1. Length distribution of particles from purified virus suspension observed by electron microscopy.

Serological properties. The antiserum produced to the maize virus had an homologous titre of 1/1024, and reacted with GGMV up to a dilution of 1/512. When tested with purified maize virus, GGMV antiserum (homologous titre 1/1024) reacted up to a dilution of 1/512 and pepper veinal mottle virus antiserum (homologous titre 1/4096) up to a dilution of 1/256. No reaction occurred between the maize virus and antisera to the following viruses (homologous titres in parentheses): bean common mosaic (1/2048), bean yellow mosaic (1/512), henbane mosaic (1/8000), maize dwarf mosaic, strain A (titre unknown), passionfruit ringspot (1/2048), peanut mottle (1/256), potato A (1/4096), potato Y (1/2048), sugarcane mosaic, strains H (1/100) and J (1/100), tobacco etch (1/152), turnip mosaic (1/64).

Insect transmission. The virus was transmitted by aphids to nine of 28 plants tested. Transmission after brief acquisition and test feeding periods suggest that transmission is of the non-persistent type. However, in similar tests, GGMV was not transmitted.

DISCUSSION

The maize virus is barely distinguishable from GGMV on the basis of symptoms induced in maize, *in vitro* properties, and particle size and morphology. Host range, aphid transmission, behaviour on electro-focusing and serological properties are however sufficiently different to justify a taxonomic distinction between the two isolates.

Of 33 graminaceous species tested, the following 10 react differently to the two viruses: *Avena fatua, A. paniculata, A. strigosa, Bromus macrostachys, Eleusine coracana, E. tocussa, Lolium multiflorum, Panicum capillare, P. maximum* 'K 187' and *Sorghum vulgare.*

Although the virus was transmitted by aphids, GGMV was not. Banding upon isoelectric focusing differs significantly between the two viruses, although the measured values cannot be taken as the isoelectric point of native particles because infectivity is lost during focusing in the

ampholine column. GGMV and maize virus antisera each reacted to a dilution close to their homologous titres with the heterologous antigen. Nevertheless, the maize virus appears to be more closely related to pepper veinal mottle virus than is GGMV, which reacted with the same pepper veinal mottle antiserum up to a dilution of only 1/64.

On the basis of the above similarities and differences between the maize virus and GGMV, we conclude that they are closely related though distinguishable. The differences in host range, serological and isoelectric focusing properties are of kinds characteristic of different strains, although the difference in transmissibility by aphids might suggest a greater difference. However, lack of aphid transmissibility occurs in strain C of potato virus Y and need not suggest a greater taxonomic divergence than of strains (Delgado-Sanchez & Grogan, 1970). So we propose to name the maize virus: GGMV strain B (GGMV-B), and consequently to rename the virus described by Thouvenel *et al.* (1976) as GGMV strain A (GGMV-A).

GGMV-B is a newly recorded naturally occurring virus on maize. Sugarcane mosaic virus was previously the only potyvirus to have been recorded infecting maize crops in Africa (Von Wechmar & Hahn, 1967; Kulkarni, 1973). Though producing identical symptoms on Gramineae, GGMV-B can be readily distinguished from sugarcane mosaic virus on the basis of host range and serological properties; particle size is also different (750 nm for sugarcane mosaic virus); however the length of particles of several potyviruses (including GGMV) differ according to the suspending medium and it is not known how the two viruses would be affected. Further work is planned to study the incidence of GGMV-B in Ivory Coast and its effect on yield and quality of maize.

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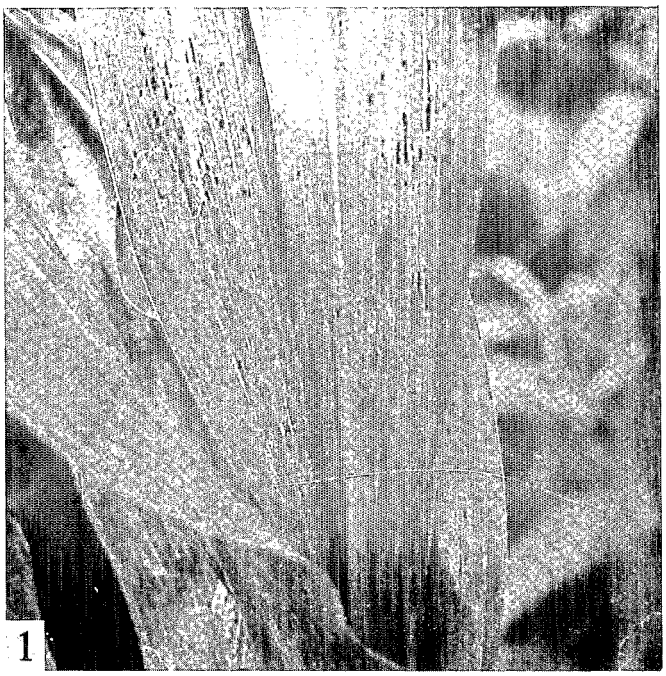
EXPLANATION OF PLATE

Fig. 1. Plant of maize naturally infected with the maize virus.

Fig. 2. Particles of purified maize virus stained with 3% uranyl acetate. Micrograph by M. Dollet. Bar represents 200 nm (some particles are the underside of the support film).



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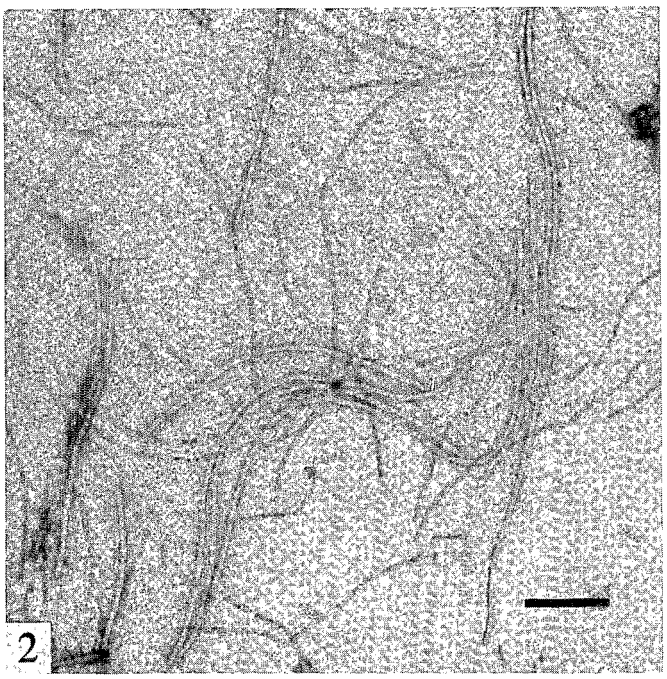

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