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Identification of a strain of maize dwarf mosaic virus, related to sugarcane mosaic virus isolated from maize in Burundi

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Summary

A strain of maize dwarf mosaic virus related to sugarcane mosaic virus has been isolated from maize in Burundi.
The properties (including electron microscopy and serology) of the virus are described, and elements for a control strategy are reviewed.

Résumé

Une variante du virus du nanisme du maïs, apparentée au virus de la mosaïque de la canne à sucre, a été identifiée sur maïs au Burundi.
Les propriétés (notamment microscopie électronique et sérologie) du virus sont décrites et des éléments de lutte sont envisagés.

Introduction

Maize is one of the important food crops in Burundi. Among the virus diseases of this species, maize streak is one of the most common. During a stay in Burundi, one of us (M.V.) observed a symptom that could be attributed to infection with a potyvirus. This paper described the identification and properties of this isolate.

Materials and methods

Samples were taken in a field near Tesa from plants with reduced growth, with leaves showing streaks of chlorotic points and mottling. Leave blades in a plastic bag for further testing were taken to our laboratory in Belgium.

Test-plants were infected by mechanical inoculation with maize extracts mixed with 300 Carborundum powder.

Seedlings of different species were tested in an insect-proof glasshouse, and symptoms were recorded.

Cuttings of healthy Sugarcane *(Saccharum officinarum)* varieties (Co 281; C.P. 29-291; C.P. 31-294; C.P. 31-588) were obtained from the quarantine station of Muguga, Kenya.

The virus was maintained during the experiments on *Zea mays*, var. 'Cargill Primeur', and subsequently in calcium chloride dessicated leaves in a cold room (2).

Thermal inactivation point, dilution end point and preservation *in vitro* were determined as described in Noordam, 1973.

Apterous non viruliferous *Myzus persicae* reared on *Vicia faba* or *Brassica napus* were used for vector transmission experiments.

Leaf exsudates were observed in electron microscopy following the leaf dip method described by Verhoyen and Creemers (16).

Ultrastructural characteristics were observed after dehydration and embedding in Epon. Thin sections were obtained with a LKB ultrotome and were stained with lead citrate and uranyl acetate, as described previously (5).

Antisera against Maize dwarf mosaic virus — strain A (MDMV-A) and strain B (MDMV-B) were obtained from Dr. Gordon (Ohio agricultural research and development center, Wooster) and antiserum PVAS-51 against surgarcane mosaic virus-strain H (SCMV-H) was from ATCC.

Microprecipitation tests were carried out on microscope slides and immunodiffusion tests with sodium dodecyl sulfate (SDS) 0,5% were performed as described by Purcifull and Shepherd (9).

Partial purification of the virus was obtained using the method described by Bond and Pirone (1).

Results

1. Host range

Mechanical inoculation of maize seedlings (var. 'Cargill primeur') resulted in symptom appearance in about ten days. Infected plants were used as inoculum source for the host range experiments.

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Table 1 shows the tested species and their susceptibility. The following species showed no symptoms and the retroinoculations were negative. Monocotyledones: *Avena byzantina, Avena sativa, Bekeropsis uniseta, Brachiaria ruziziensis, Coix lacryma-jobi, Cynodon dactylon, Dactylis glomerata,* var. *'Lemba', Eleusine indica, Festuca ovina, Festuca pratensis,* var. *'Merbeen', Hordeum vulgare,* var. *'Capri', Lolium multiflorum,* var. *'Italicum', Lolium multiflorum,* var. *'Westerworld', Lolium perenne, Oryza barthii, Oryza stativa,* var. *'IRAT 11', Oryza stativa,* var. *'IR 8', Panicum miliaceum, Penisetum clandestinum, Penisetum purpureum, Penisetum saliflex, Phleum pratense, Poa communis, Poa pratensis,* var. *'Prato', Saccharum spontaneum, Saccharum officinarum,* var. 'CP 31-558', *Saccharum officinarum,* var. 'CP 29-291', *Sorghum halepense, Sorghum vulgare* hybrid AKS 653, *Sorghum vulgare* hybrid AKS 663, *Sorghum vulgare,* var. *'Sudanense', Triticum aestivum* ssp. *vulgare;* Dicotyledones: *Allium porrum, Ammi majus, Apium graveolens; 'rapaceum', Brassica napus,* var. *'napus', Chenopodium quinoa, Cichorium endivia,* var. *'Latifolia', Cucumis melo, Cucurbita maxima, Datura stramonium, Dianthus chinensis, Lycopersicon esculentum, Melinis minutiflora, Nicotiana tabacum, Phaseolus vulgaris, Pisum sativum, Solanum melongena, Spinacia oleracea,* var. 'Nores'.

Table 1

Susceptibility of the species tested against the virus infection
Susceptibilité des espèces végétales testées vis-à-vis du virus

Tested species	Symptoms	Symptoms after retro-inoculation on *Zea mays*
Echinochloacrus-galli	M	M(5/6)(7/9)
Oryza sativa var. 'IR 442'	O	M (6/6)
Setaria italica	M	M (4/6)
Saccharum officinarum, var. 'Co 281'	Chl.str.	M (7/8)
Saccharum officinarum, var. 'CP 31 294'	Chl.sp.	M(8/8)(3/9)
Saccharum officinarum, var. 'Wild type'	Chl.str	M (3/8)
Sorghum bicolor, var. 'Atlas'	M	—
Sorghum bicolor, var. 'Rio Sorgo'	M	—
Sorghum bicolor, var 'Sart'	M	—
Zea mays, var. 'Cargill Primeur'	M	100%
Zea mays, var. 'But 234'	M	100%
Zea mays, var. 'Aurelia'	M	100%
Zea mays, var 'Fronica'	M	100%
Zea mays, var 'Royal 255'	M	100%
Zea mays, var. 'LG 7'	M	100%

Legend: O: no symptoms *(pas de symptômes)*
M: mosaic *(mosaique)*
Chl. str.. chlorotic streaks *(striures chlorotiques)*
Chl. sp.. chlorotic spots *(taches chlorotiques)*
In brackets (number of infected plants/ number of inoculated plants)
Entre parenthèses (nombre de plantes infectées/nombre de plantes inoculées)

2. Symptomatology

Zea mays: when plants were mechanically inoculated at the two leaf stage, the first symptoms appeared on the new leaves after about 5-6 days. Inoculated leaves showed no symptoms. With further development of the leaves, chlorotic spots became brighter and were dispersed as streaks along the veins. On mature leaves, chlorosis appeared as streaks or mosaic. Growth of infected plants was less developed than the healthy ones.

Echinochloa crus-galli: the first symptoms appeared on the leaves newly formed after inoculation; chlorosis in patches formed on the leafblades.

Oriza sativa: no symptom appeared on this species, but retroinoculation of var. 'IR 442' showed that the virus was latent in this species.

Setaria italica, Sorghum bicolor: the symptoms resembled those on *Zea mays* and appeared respectively 12 and 14 days after inoculation on the newly formed leaves.

Saccharum officinarum: symptoms appeared on newly formed leaves; chlorotic streaks and bands of dark green tissue developped on the expanded leaves. No symptoms appeared on the leaves already formed before inoculation.

3. Properties of the virus isolate in maize plant sap

The virus persisted for 10 min. at 50°C but is inactivated at 55°C.

Two dilution end point assays were made; the first with plant extracts prepared 4 weeks after inoculation, the second after 8 weeks. In the two experiments plant sap was still infective at a dilution of 5.10^{-4} but not at 1.10^{-4}.

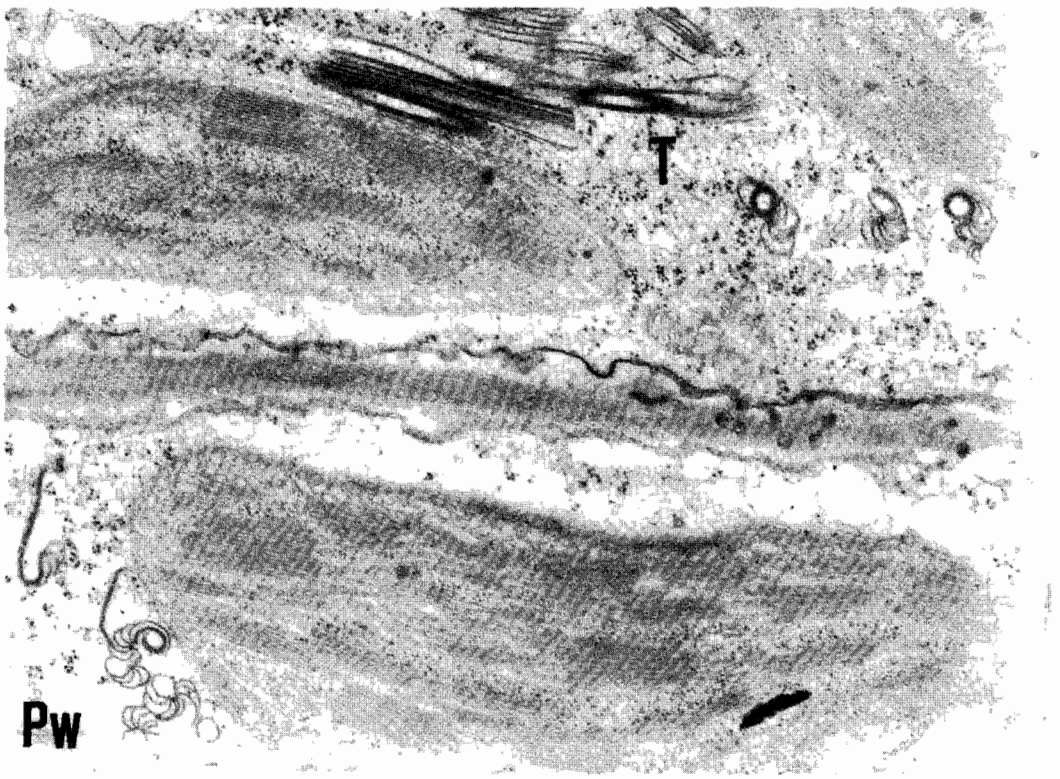
The virus was still infective after 24 hours at room temperature but not after 36 hours. At 4°C, infectivity lasted for 60 hours, but not for 72 hours. In frozen tissue kept at -18°C, the virus was still infective after 50 days. The virus was still infective after 10 months in tissue dessicated over Calcium chloride.

Apterous non viruliferous *Myzus persicae* were starved for 2 hours before an acquisition feeding period of 5 and 10 min. on diseased maize leaves, and were then transferred to healthy maize plants for 24 hours; 4 plants and 2 plants, respectively, showed symptoms within inoculated plants.

Leaf dips showed elongated particles with a mean lenght of 711 nm and 708 nm, respectively in two experiments.

Ultrathin sections in leaf cells showed pinwheel structures and bundles of tubes in the cytoplasm (fig. 1).

Microprecipitin tests showed positive reactions between extracts of infected maize and MDMV-A,



Pw Pinwheel
T Tubes

Grossissement X 33 000

Figure 1 Ultrathin section in a mesophyll portion of maize leaf infected with the virus isolate
Coupe ultramince dans une portion de mésophylle de feuille de maïs infectée par le virus isolé

MDMV-B or SCMV-H antisera. Immunodiffusion tests showed that the virus was related to the MDMV-A and MDMV-B

The virus isolate could be easily concentrated and partially purified, following the method described by Pirone and Anzalone (8), as shown in figure 2.

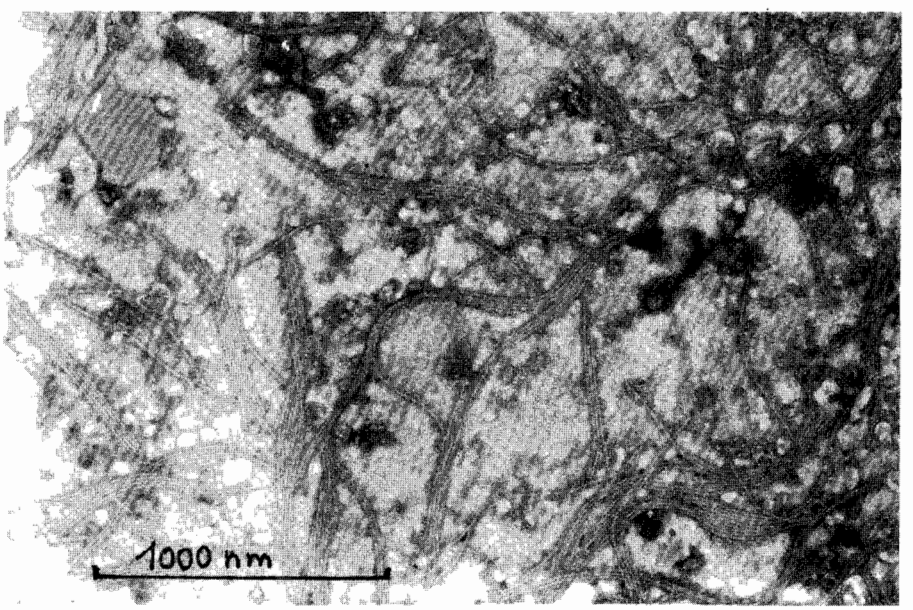


Figure 2 Partially purified virus particles
Particules de virus partiellement purifiées.

Conclusions

The virus, we isolated from maize in Burundi, was not related to the already known maize streak virus. This 700 nm long virus forming pinwheel inclusions belongs to the potyvirus group. Five potyviruses have been described on Maize: sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), maize mosaic virus (MVV), sorghum red stripe virus (SRSV) and ragi disease complex (eleusine mosaic virus). These viruses are all related to SCMV. Grancini and Mariani (4) identifying a SCMV in *Sorghum* concluded, with Snazelle *et al.* (13), 'that much confusion still remains as how these viruses are related'. Many strains exist in different countries: SCMV — A,B,C,D^1,D^2,D^3,E,F,G; MDMV — A,B; MMV — 1,2,3. Several criteria were used to differentiate these strains: host range in terms of plant species (7;10), in sugarcane (14) or sorghum (12) varieties and serology (3).

The maize virus of Burundi reacted with antiserum MDMV-A, MDMV-B and SCMV-H, so that we may conclude that it is a MDMV strain related to SCMV. Further experiments have to be carried out to classify the isolated strain and to clarify the confusion existing in the literature about these strains (4)

The important practical conclusion for Burundi is that maize is infected not only with maize streak virus, a geminivirus transmitted by leafhoppers, but also with an elongated non persistent aphidborne virus, related to sugarcane mosaic virus which may also be seed-borne in maize (15).

This calls for specific control measures based on SCMV host range and vector relationship including selection, as resistant lines do exist (11).

References

(1) Bond, W.P. and Pirone, T.P., 1971 Purification and properties of sugarcane mosaic virus strains. Phytopathol. Z.,**71:** 65-64.

(2) Bos, L., 1969. Experience with a collection of plant viruses in leaf material dried and stored over calcium chloride, and a discussion of literature on virus preservation. Meded. Fak. Landbouwwetensch. Gent, **34**: 875-887.

(3) Gordon, D.T. and Williams, L.E., 1970. The relationship of a maize virus isolate from Ohio to Sugarcane mosaic virus strains and the B strains of Maize dwarf mosaic virus. Phytopathology, **60**: 1293 (abstr.).

(4) Grancini, P. and Mariani, G., 1974. Sugarcane mosaic virus in Sorghum. Mikrobiologya, **11**: 9-17.

(5) Horvat, F. and Verhoyen, M., 1973. La striure chlorotique du poireau. I. Identification de l'agent causal. Parasitica, **29**: 16-28.

(6) Noordam, D., 1973. Identification of plant viruses. Methods and experiments. Pudoc, Wageningen, pp 207.

(7) Paliwal, Y.L., Raychaudhury, S.P and Renfro, B.L., 1968. Some properties and behaviour of Maize mosaic virus in India. Phytopathology, **58**: 1682-1684.

(8) Pirone, T.P. and Anzalone, L., 1966. Purification and electron microscopy of Sugarcane mosaic virus. Phytopathology, **56**: 371-372.

(9) Purcifull, D.E., and Shepherd, R.J., 1964 Preparation of protein fragments of several rod-shaped plant viruses and their use in agar-gel diffusion tests. Phytopathology, **54**: 1102-1108.

(10) Rosenkranz, E., 1977. Grasses native or adventive to the United States as new hosts of Maize dwarf mosaic and Sugarcane mosaic viruses. Phytopathology, **68**: 175-179.

(11) Scott, G.E., and Rosenkranz, E., 1981 Effectiveness of resistance to Maize dwarf mosaic and Maize chlorotic dwarf viruses in Maize. Phytopathology, **71**: 937-941.

(12) Sehgal, O.P., Jean, J. and Zuber., 1968. Sorghum hybrids as local lesion hosts for Maize dwarf mosaic virus. Phytopathology, **58**: 1708-1709.

(13) Snazelle, T.E., Bancroft, J.B. and Ullstrup, A.J., 1971. Purification and serology of Maize dwarf mosaic and Sugarcane mosaic viruses. Phytopathology, **61** 1059-1063.

(14) Summers, E.M., 1935. Strains of the Sugarcane mosaic virus in Louisiana. Phytopathology, **24**: 1040-1042.

15) Tosic, M. and Sutic, D., 1977 Investigation of Maize mosaic virus transmission through corn seed. Ann. Phytopathol., **9**: 403-405.

(16) Verhoyen, M. and Creemers, J., 1969. Presence of the different components and of particle aggregates of Alfafa mosaic virus in crude extracts from infected *Nocitiana tabacum*. Phytopathol. Zeitschrift, **65**: 291-294.

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