Isolation and Purification of a Virus Transmitted from Mosaic-Diseased Cassava in the Ivory Coast

B. WALTER, Laboratoire de Virologie, Centre de Recherches d'Adiopodoumé, Office de la Recherche Scientifique et Technique Outre-Mer, Abidjan, Côte d'Ivoire, Africa

ABSTRACT

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A virus was mechanically transmitted from cassava leaves with typical symptoms of the African cassava mosaic disease to *Nicotiana benthamiana* and to hybrid *N. clevelandii* × *N. glutinosa*, and from *N. benthamiana* to other solanaceous species. The virus was purified and appeared as geminated particles in electron micrographs. This is the first report of a geminivirus in mosaic-diseased cassava in West Africa.

Since African cassava mosaic disease was first reported in 1894 (14), it has been observed in all parts of East, West, and Central Africa and the adjacent islands (6,8,9). The disease was first reported in the Ivory Coast by Hedin in 1931 (11). In the coastal areas of the Ivory Coast, all cassava (*Manihot esculenta* Crantz) cultivars show severe symptoms, while in the northern part of the country, many affected cultivars are symptomless during the dry season (7).

Several attempts have been made to purify the causal agent(s) without success. We report the isolation of a virus transmitted from cassava collected near Abidjan, Ivory Coast.

MATERIALS AND METHODS

Transmission. Young leaves from *M. esculenta* or *M. glaziovii* plants with severe symptoms of mosaic, distortion, and reduction in size were ground with buffer (5–10 ml/g). Several buffers were tested: borate, phosphate, citrate-phosphate, hepes (N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid), and tris-HCl, at concentrations ranging from 0.004 to 0.5 M, pH from 6.0 to 9.0, with or without additives (2-mercaptoethanol, thioglycolic acid, sodium disulfite).

Carborundum-dusted leaves of *N. benthamiana* and *N. clevelandii* × *N. glutinosa* were inoculated with the crude sap. The test plants were grown in insect-proof greenhouses under local climatic conditions (mean temperature 28 C, average minimum relative humidity 65%). Leaves of diseased *N. benthamiana* were used as the source of inoculum for mechanical transmission to the following plants: Aizoaceae—*Tetragonia expansa*; Amaranthaceae—*Celosia argentea, Gomphrena globosa*; Chenopodiaceae—*Beta vulgaris, Chenopodium amaranticolor, C. capitatum, C. murale, C. quinoa*; Convolvulaceae—*Ipomoea mauritiana*; Cucurbitaceae—*Cucumis melo, C. sativus*; Euphorbiaceae—*Jatropha gossypifolia, Ricinus communis*; Leguminosae—*Arachis hypogaea, Cajanus cajan, Phaseolus lathyroides, P. vulgaris, Psophocarpus tetragonolobus, Vigna sinensis, V. unguiculata;* Scrophulariaceae—*Antirrhinum majus*; Solanaceae—*Capsicum annuum, C. frutescens, Datura stramonium, Nicotiana acuminata, N. affinis, N. bigelovii, N. clevelandii, N. debneyi, N. forgetiana, N. glutinosa, N. knightiana, N. longiflora, N. megalosiphon, N. occidentalis, N. repanda, N. rustica, N. suaveolens, N. sylvestris, N. tabacum, Petunia hybrida, Physalis aequata, P. alkekengi, P. peruviana, Solanum melongena, S. nigrum.*

Properties in vitro. *N. benthamiana* plants were used as the inoculum source and the systemic assay host. The dilution end point was determined by serial dilutions of crude sap in 0.2 M phosphate buffer, pH 7.6. The thermal inactivation point was tested by immersing 2-ml samples of crude sap in a water bath at a given temperature for 10 min and then in an ice bath. To test in vitro aging, crude extracts were kept at 4 C and at room temperature.

Purification and electron microscopy. Leaves from *N. benthamiana* or *N. glutinosa* × *N. clevelandii* (100 g) were harvested 3–4 wk after inoculation and homogenized in 300 ml of 0.2 M phosphate buffer containing 0.004 M ethylene diamine tetraacetate (EDTA) and 1% 2-mercaptoethanol, pH 7.8. The homogenate was squeezed through nylon, acidified to pH 4.4, kept 30 min at 4 C, and readjusted to pH 7.8 (13).

After centrifugation at 1,500 *g* for 5 min and at 8,000 *g* for 10 min in a Sorvall RC2-B centrifuge with a GSA rotor, ammonium sulfate was added to the supernatant fluid at a concentration of 30 g/100 ml. Precipitates were pelleted by centrifuging at 1,500 *g* for 5 min and at 16,000 *g* for 10 min and were resuspended in 50 ml of 0.02 M phosphate buffer containing 0.004 M EDTA, pH 7.6. After a low-speed centrifugation, the supernatant was centrifuged at 82,500 *g* for 3 hr through a 12-ml 20% (w/v) sucrose cushion in a Beckman L5-50 centrifuge with an SW 25-2 rotor. The final pellet was resuspended in 2 ml of the resuspension buffer.

The purified virus preparation (1.2 ml) was loaded on 4 ml of a 50% (w/v) cesium chloride solution in 0.02 M phosphate buffer, pH 7.6, in a Beckman SW 50L rotor tube and centrifuged at 130,000 *g* for 8–16 hr. Densities of cesium chloride solutions were calculated from refractive index measurements with a refractometer as reported by Bruner and Vinograd (4).

Virus preparations were stained with 2% aqueous uranyl acetate, pH 5, and observed at 80 kV with a Siemens Elmiskop 102 electron microscope at the Groupe d'Etudes et de Recherches en Microscopie Electronique at Adiopodoumé.

RESULTS

Transmission. Highest percentage transmission (100%) was obtained with 0.01 and 0.1 M buffers at about pH 8.0. Transmission also occurred in distilled water. Leaf rolling, crinkling, and occasional local chlorotic lesions near the

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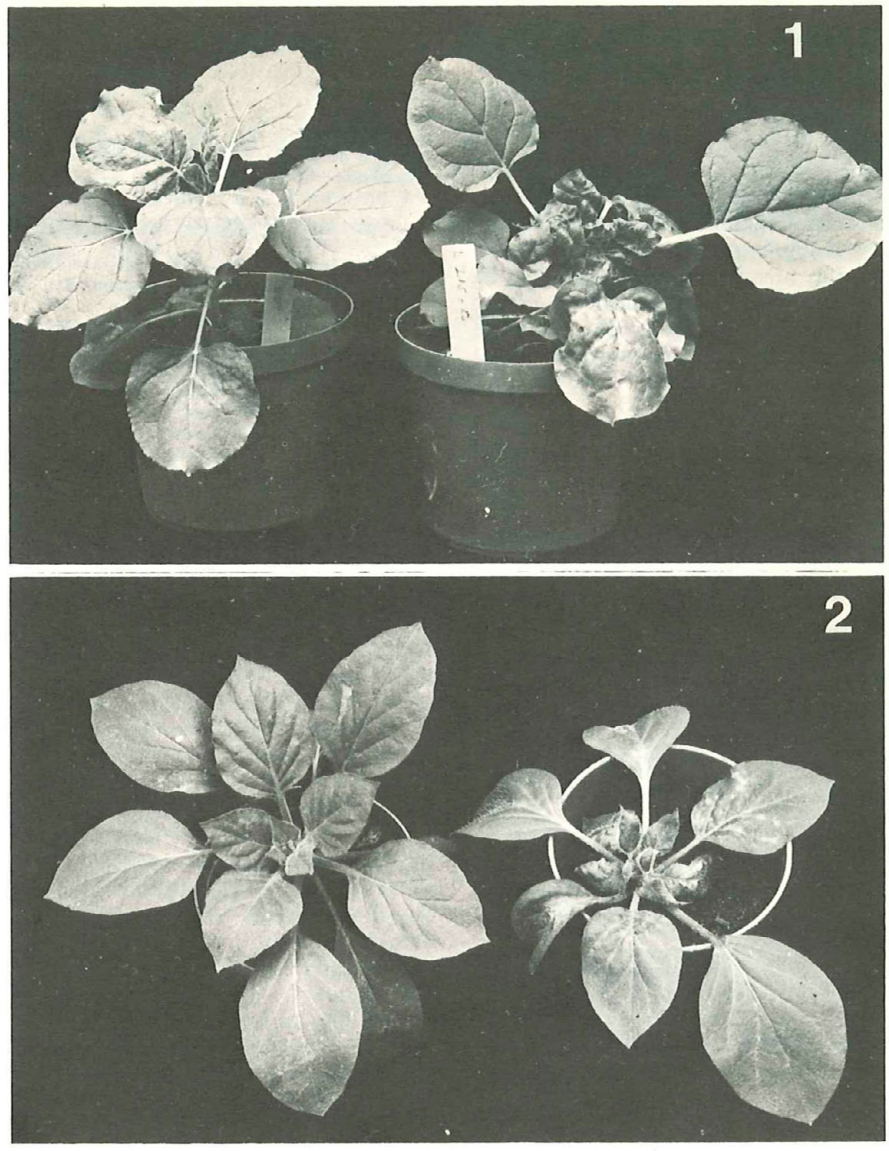
veins appeared on *N. benthamiana* 10 days after inoculation with crude sap from *M. esculenta* or *M. glaziovii*. Later, yellow areas developed on the leaves, and the top of the plant showed curling and stunting (Fig. 1). Similar symptoms were observed on *N. glutinosa* × *N. clevelandii* (Christie's Hybrid) (Fig. 2).

The species that became infected when inoculated with an extract from diseased *N. benthamiana* were *N. clevelandii* (principal symptoms leaf curl, rugosity, and yellow leaf areas); *N. glutinosa* (stunting, leaf curl, and deformation); *N. forgetiana* (vein banding, mottling, and leaf deformation); *N. sylvestris* (mottling, yellow areas, and deformation of the plant top); and *N. tabacum* (rugosity and distortion of the upper leaves). Mechanical transmissions from these hosts and from the hybrid *N. glutinosa* × *N. clevelandii* back to *N. benthamiana* were positive. The remaining species tested could not be infected, as indicated by lack of symptoms on *N. benthamiana* after back-inoculation with sap from these species.

Properties in vitro. The thermal inactivation point of the virus was between 45 and 50 C. Infectivity of the virus in sap was lost in 5–6 days at room temperature and in 13–17 days at 4 C. Dilution end point of the virus was between 10^{-3} and 5×10^{-4}.

Purification and electron microscopy. When centrifuged in cesium chloride gradients for 16 hr at 130,000 *g*, the virus was concentrated as a sharp light-scattering band in the lower third of the tube at a density of 1.35 ± 0.01 g/cm^3. This zone was highly infectious on *N. benthamiana*. The ultraviolet absorption spectrum of this fraction showed a maximum at 258–260 nm and a minimum at 238–240 nm with an A_{260}/A_{280} ratio of 1.55 ± 0.10.

The infective fraction from cesium chloride gradients contained numerous isometric particles about 20 nm in diameter that were often paired (Fig. 3).



Figs. 1–2. Typical symptoms on **(1)** *Nicotiana benthamiana* and **(2)** hybrid *N. glutinosa* × *N. clevelandii* after mechanical inoculation with crude sap from mosaic-diseased cassava. Healthy controls at left.

DISCUSSION

The virus purified from *N. benthamiana* originally inoculated with sap from mosaic-diseased cassava belongs to the group for which the name geminivirus has been proposed (10). Members of this group have unusually small isometric particles, 15–20 nm in diameter, that occur mostly in pairs.

The virus described here is similar to that named cassava latent virus by Bock et al in Kenya (3), except that we could not reproduce under our conditions the symptoms on *N. benthamiana* when back-inoculated with sap from previously inoculated *Datura stramonium*, *N. debneyi*, *N. rustica*, and *Solanum nigrum*, even though these plants showed slight mottling and deformation. *N. glutinosa* × *N. clevelandii*, *N. forgetiana*, and *N. sylvestris* are new hosts. A geminivirus was also isolated from mosaic-diseased cassava at the International Institute of Tropical Agriculture (IITA) in Nigeria (1). These results suggest that the same virus is present in all mosaic-diseased cassava in Africa.

The fact that the virus was never isolated from cassava plants without typical mosaic symptoms and that we could isolate the virus from wild cassava (*M. glaziovii*) suggests that this geminivirus may be the causal agent of the mosaic disease. Transmission back to cassava is necessary to confirm this hypothesis; however, to date all attempts to infect healthy cassava with the purified geminivirus mechanically or with the natural vector *Bemisia tabaci* have failed. We have also tried without success graft transmission from tobacco to cassava by approach or by aseptically "hetero-grafting" cuttings in vitro.

These failures may be due to difficulties in infecting cassava, or the geminivirus may not be the causal agent or the sole causal agent of the mosaic disease. Contradictory results have been reported concerning transmission from cassava to cassava, and early reports of experimental transmission by injection of infective sap in leaf parenchyma (12) or by incision of the stem (11) could not be confirmed.

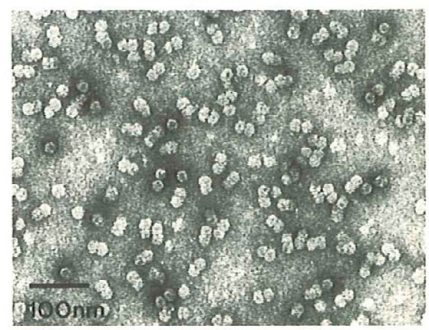


Fig. 3. Virus particles negatively stained in uranyl acetate. Note the association of the particles in doublets.

Recently, transmission was accomplished by standard methods of sap inoculation of plant viruses, using South American hypersensitive cultivars (2), which indicates that there are no strongly inhibitory systems in cassava sap.

The African cassava mosaic disease belongs to the group of whitefly-vectored plant diseases that have been difficult or impossible to transmit mechanically (5), possibly because the causal agent may be restricted to internal phloem tissues or because the causal agent and/or host are sensitive to the stress of routine mechanical transmission techniques.

LITERATURE CITED

1. ANONYMOUS. 1979. Research Highlights for 1978. International Institute of Tropical Agriculture, Ibadan, Nigeria. Pages 25-29.
2. BOCK, K. R., and E. J. GUTHRIE. 1978. Transmission of African cassava mosaic by mechanical inoculation. Plant Dis. Rep. 62:580-581.
3. BOCK, K. R., E. J. GUTHRIE, and G. MEREDITH. 1978. Distribution, host range, and purification of cassava latent virus, a geminivirus. Ann. Appl. Biol. 90:361-367.
4. BRUNER, R., and J. VINOGRAD. 1965. The evaluation of standard sedimentation coefficients of sodium RNA and sodium DNA from sedimentation velocity data in concentrated NaCl and CsCl solutions. Biochim. Biophys. Acta 108:18-29.
5. COSTA, A. S. 1976. Whitefly-transmitted plant diseases. Annu. Rev. Phytopathol. 14:429-449.
6. DEIGHTON, F. C. 1927. Mycological Section, Annual Report for the Year 1926. Lands and Forest Dep., Sierra Leone.
7. DUBERN, J. 1976. La mosaïque du manioc: Bilan des connaissances actuelles. Rapport ORSTOM. 29 pp.
8. DUFRENOY, J., and L. HEDIN. 1929. La mosäique des feuilles du manioc au Cameroun. Rev. Bot. Appl. Agric. Colon. 9:361.
9. HALL, F. W. 1928. Annual Report. Dept. Agric., Uganda. Government Printer. Entebbe.
10. HARRISON, B. D., H. BARKER, K. R. BOCK, E. J. GUTHRIE, G. MEREDITH, and M. ATKINSON. 1977. Plant viruses with circular single-stranded DNA. Nature (London) 270: 760-762.
11. HEDIN, L. 1931. Culture du manioc en Côte d'Ivoire: Observations complémentaires sur la mosäique. Rev. Bot. Appl. Agric. Colon. 11:558.
12. LEFEVRE, P. 1935. Quelques considérations sur la mosäique du manioc. Bull. Agric. Congo Belge 26:442-447.
13. THOUVENEL, J. C., C. FAUQUET, and M. DOLLET. 1978. Influence du pH sur le virus du clump de l'arachide (peanut clump virus) et nouvelle méthode rapide de purification. Ann. Phytopathol. 10:93-99.
14. WARBURG, O. 1894. Die Kulturplanzen Usambaras. Mitt. Dtsch. Schutzgeb. 7:131.